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STUDIES ON NORTH AMERICAN HELMINTHS OF THE GENUS *CAPILLARIA* ZEDER, 1800 (NEMATODA): I. CAPILLARIDS FROM MAMMALS*

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Most of the material on which the present study is based was collected by Dr. Robert Rausch of the Department of Veterinary Science, University of Wisconsin, who kindly made the material available for study. Some additional material was collected by the writer near Houston, Texas. My thanks are due to Dr. Asa C. Chandler for helpful suggestions and criticism.

The genus *Capillaria* Zeder, 1800, contains many species which are inadequately described, as has been pointed out by Travassos (1915), Teixeira de Freitas and Lent (1936), and other authors. Because of this and because many species have been described from only a few specimens, it is almost impossible to determine the limits of variation of many members of the genus and any comprehensive study of the group is rendered difficult. A thorough restudy of the genus is needed, but it is probable that such a task could better be undertaken by some worker with access to large collections of material, such as that of the U. S. National Museum, or some of the larger European institutions.

Representatives of the genus have been reported as parasites of the alimentary canal, respiratory system, genito-urinary tract, and subcutaneous tissues of various North American mammals. A study of the literature reveals that ten or perhaps eleven species are known from North American mammals. Several new species will be described in this and forthcoming parts of this study; also, records of new hosts and localities are included.

Certain species of the genus *Capillaria* show little host specificity. *Capillaria hepatica* (Bancroft, 1893) Travassos, 1915, furnishes an excellent example of this lack of specificity. This species has been reported from a number of species of rodents, including rats, mice, prairie dogs, muskrats, beaver, and the European hare. Nishigori (1925) and Wright (1930) have reported this worm from the dog. Foster and Johnson (1939) have reported it from the liver of the peccary, the spider monkey, and the capuchin monkey. A single authentic case of human infection, a British soldier in India, has been reported by MacArthur (1924). Some other species of *Capillaria* probably exhibit a similar lack of specificity, though to a somewhat less marked degree. The fact that *C. hepatica* causes a grossly perceptible pathology of the liver may explain the fact that the hostal relations of this species

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are somewhat better known than is the case with most other members of the genus. Capillarids are probably overlooked rather frequently in parasitological examinations. The worms are small and show little movement when removed from the animal body to water or saline. Those species inhabiting the alimentary canal are often found only when the mucosa is thoroughly scraped. Data to be presented in this and later papers of the same series, afford further indications that a considerable number of species of *Capillaria* lack host specificity.

On the other hand, capillarids are somewhat more specific in regard to their location in the host animal. A pertinent example may be cited: In the domestic fowl *Capillaria collaris* (v. Linstow, 1873) [= *C. retusa* (Railliet, 1895)] is invariably found in the cecum while *C. caudinflata* (Molin, 1858) is always found in the small intestine. Although *C. caudinflata* may be found in the terminal region of the small intestine in extremely heavy infections, these two species are always in the locations noted above, though both may occur in the same host animal.

Thus, it would seem that the finding of a capillarid in the same host species as a previously described member of the genus is incomplete evidence for assuming that the worms are specifically identical; the location in the host animal is of equal, if not more, significance in making a diagnosis.

Description or discussion of five species of *Capillaria* follows:

Capillaria linearis (Leidy, 1856)

This helminth was not encountered during the course of this study. However, it has been called to the attention of the writer that this species has been subjected to a rather curious misdescription by various workers. Leidy (1856) gave a fragmentary description of *Trichosomum lineare* from the intestine of a cat. He gave the lengths of the male and female as 1½ and 3 inches respectively. Neveu-Lemaire (1912), Baylis (1929), and Teixeira de Freitas and Lent (1936) gave the lengths of the male and female of Leidy's worms as 3.8 and 7.6 mm. These measurements, evidently due to the misplacing of a decimal point in converting from inches to millimeters, are ten times smaller than the measurements given by Leidy. A search of the literature has revealed no correction of Leidy's original description by that author, and no mention of a correction has been made by other authors. As characterized by Leidy, *C. linearis* is much larger than any other North American species.

Lewis (1927) reported *C. linearis* from the urinary bladder of cats in Wales. Lewis' specimens and *Capillaria* sp. reported from the urinary bladder of Canton cats by Chen (1934) are probably referable to *C. felis-cati* (Diesing, 1851) Travassos, 1915, rather than to *C. linearis*. It seems highly unlikely to the writer that these specimens from the urinary bladders of cats are co-specific with Leidy's *Trichosomum lineare* from the intestine.

Capillaria hepatica (Bancroft, 1893) Trav., 1915.

This cosmopolitan species is here reported for the first time from the cotton rat, *Sigmodon hispidus,* taken at Houston, Texas.

Capillaria putorii (Rudolphi, 1819) Trav., 1915.

Sprehn (1932) included the U. S. in the geographical distribution of this species. The writer has been unable to locate any report of this worm from a North American

mammal and has failed to find it in any of the material examined during the course of this study. It has been reported from at least eight species of mustelids in Europe and it would not be surprising to find this worm in the North American fauna. It is possible that some of these European records of *C. putorii* were actually *C. mustelorum* Cameron and Parnell, 1932, described from mustelids in Scotland, which resembles *C. putorii* closely. Petrow (1928) redescribed *C. putorii,* and Christensen, Olsen, and Roth (1946) have recently reported it from a Copenhagen cat.

Capillaria mustelorum Cameron and Parnell, 1932.
(Plate I, Figs. 6–9)

Worms of this species were recovered from the small intestine of two of eight weasels, *Mustela frenata novaboracensis,* at East Lansing, Michigan, and from one of thirty-six mink, *Mustela vison,* at Horicon Marsh, Wisconsin, and from one of two mink at Houston, Texas. This species has not been previously reported from North America. *Capillaria* spp. reported from *Mustela vison* in North America by Law and Kennedy (1932), Allen (1934), Sealander (1943), and Erickson (1946) probably belong to this species. Since the material at hand shows a range of variation not covered in the description of Cameron and Parnell, a description of the worms seems warranted.

Description: Bacillary lines not visible. Cuticle transversely striated. Mouth simple.

Female: 4.3 to 12.1 mm long; 45 to 54 μ wide just posterior to vulva. Esophagus 2.5 to 3.3 mm long. Vulva slightly posterior to termination of esophagus, without appendages, sometimes marked by cuticular swellings which are presumably artifacts caused by fixation. Vulva divides body 1 : 1.1 to 1 : 2.6. Ova 52 to 60 by 24 to 30 μ, outer shell smooth, prominent plug at each end. Posterior extremity rounded. Anus subterminal.

Male: 3.6 to 8.0 mm long; 33 to 38 μ wide. Esophagus 1.7 to 3.7 mm long. Spicule 290 to 406 μ long, 3.8 to 4.5 μ wide near proximal end; spicule sheath transversely striated. Cauda provided with lateral cuticular alae, 98 to 120 μ long. Body terminated by two stout papillae, supporting membranous cuticular bursa. Termination of esophagus divides body 1 : 1.1 to 1 : 1.3.

Certain features serve to differentiate this species from *C. putorii* (Rudolphi, 1819) Trav., 1915. Female worms of the two species differ in the position of the vulva and in the size of the eggs. The males differ in the length of the spicule and in the relative lengths of the esophagus. Teixeira de Freitas and Lent (1936) mentioned the possible synonymy of *C. mustelorum* with *C. erinacei* (Rudolphi, 1819). The vulva of *C. erinacei,* as described by various workers, differs from that of *C. mustelorum* in possessing markedly salient labia; the cuticular inflations resulting from fixation sometimes seen in females of *C. mustelorum* may cause confusion of the two species. The spicule of *C. erinacei* has not been adequately described and, thus, cannot furnish a distinctive character for the separation of the two species.

C. mucronuta (Molin, 1858), an insufficiently described species from the urinary bladder of *Martes foina* in Europe, is probably a valid species although it is certainly similar to the three forms just mentioned.

Capillaria rauschi n. sp.
(Plate II, Figs. 10–16)

Specific diagnosis: Bacillary lines not visible. Mouth simple. Cuticle finely striated transversely.

Female: 5.2 to 7.4 mm long; 48 to 53 μ wide just posterior to vulva; maximum width 61 to 68 μ. Vulva slightly posterior to termination of esophagus, 2 mm from anterior end, slightly

salient or with campanuliform appendage. Vulva divides body 1 : 1.6 to 1 : 1.9. Ova 59 to 62 μ by 26 to 28 μ, with outer shell mammillated and with inner shell forming a collar around the opercular plug at one end. Anus subterminal.

Male: 4.8 mm long; maximum width 50 μ. Esophagus 1.98 mm long. Spicule sheath transversely striated; spicule 395 μ long and 8 μ wide near proximal end, which is widened and appears bilobate in lateral view. Body terminated by two stout bilobed papillae supporting a membranous cuticular bursa. Cloacal opening subterminal at base of caudal papillae. Termination of esophagus divides body 1 : 2.4.

Host: Sorex cinereus Kerr.

Habitat: Small intestine.

Locality: Madison, Wisconsin.

Type: U. S. National Museum, Helminth. Coll. No. 46357.

Three females and one male of this species were recovered from a single masked shrew. This species differs from *C. splenaeca* (Dujardin, 1843) Travassos, 1915, described from *Sorex araneus* in Europe, and from *C. minutus* Chen, 1937, from *Suncus coerulus* in China, in size of eggs and in lack of lateral caudal alae. The mammillated outer shell of the ova will differentiate *C. rauschi* from most species reported from mammals.

The species is named in honor of Dr. Robert Rausch, previously mentioned as the collector of this material.

Capillaria tamias-striati n. sp.

(Plate I, Figs. 1–5)

Specific diagnosis: Bacillary lines not visible. Mouth simple. Body finely striated transversely.

Female: 11.8 to 14.4 mm long; 45 to 50 μ wide just posterior to vulva. Vulva slightly posterior to termination of esophagus, 3.0 to 4.6 mm from anterior extremity, and marked by pronounced cuticular thickenings. Vulva divides body 1 : 2 to 1 : 3. Ova 53 to 57 μ long by 25 to 27 μ wide; outer shell with reticulate markings; inner shell thin and forming collar at each end around opercular plugs. Posterior extremity straight and conical. Anus subterminal.

Male: 6.0 to 7.6 mm long; 42 to 48 μ wide in posterior region. Esophagus 2.7 to 3.1 mm. long. Spicule sheath striated coarsely in distal portion and finely striated or not striated in proximal portion, 41 to 44 μ wide in proximal portion. Spicule finely striated, 490 to 502 μ long by 7 to 8 μ wide at base; about 26 μ from distal tip spicule abruptly narrows to width of 3 μ. Cauda provided with lateral cuticular alae, 110 to 160 μ long; fine transverse ribs sometimes visible on alae. Body terminated by two stout blunt projections, supporting a membranous cuticular bursa. Cloacal opening subterminal. Termination of esophagus divides body 1 : 1.2 to 1 : 1.4.

Host: Tamias striatus L.

Habitat: Small intestine.

Locality: Madison, Wisconsin.

Type: U. S. National Museum, Helminth. Coll. No. 46358.

Worms of this species were recovered from two of forty-three chipmunks examined at Madison. This worm bears a striking resemblance to *C. erinacei* (Rudolphi, 1819), described and reported from European hedgehogs; however, there are certain rather minute differences which seem to warrant referring this material to a new species. The vulva of *C. erinacei* is described as being redundant, and the figure given by Eberth (1863) of *Trichosomum exiguum* Duj., 1845, [= *C. erinacei* (Rud., 1819)] shows the labia as prominent structures quite unlike the pre- and postvulvar cuticular thickenings of *C. tamias-striati.* Both Eberth (1863) and v. Linstow (1878) described and figured the caudal papillae of the male of *C. erinacei* as two slender finger-like structures. In contrast, the male of *C. tamias-striati* possesses rather stout papillae. In addition, *C. erinacei* possesses lateral bacillary lines which are about one-third the diameter of the body in width. Bacillary lines

were not seen by the writer in any specimens of *C. tamias-striati,* although thirty-one were available for study. It should be noted that in some males of *C. tamias-striati* fine, transverse, rib-like ridges were seen on the caudal alae. Since specimens not showing this feature differed significantly in no other way from males in which the ribs were plainly visible the writer must assume that this variability is probably due to differences in fixation.

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Explanation of Plates

All drawings made with the aid of a camera lucida.

Plate I.

Capillaria tamias-striati

Fig. 1. Vulvar region, lateral.
Fig. 2. Cauda of male, lateral.
Fig. 3. Cauda of male, ventral.
Fig. 4. Spicule, proximal end.
Fig. 5. Egg.

Capillaria mustelorum

Fig. 6. Cauda of male, Lateral.
Fig. 7. Vulvar region, lateral.
Fig. 8. Vulvar region, lateral.
Fig. 9. Egg.

Plate II.

Capillaria rauschi

Fig. 10. Cauda of male, dorsal.
Fig. 11. Cauda of male, latero-ventral.
Fig. 12. Spicule, lateral view of proximal end.
Fig. 13. Egg.
Fig. 14. Cauda of female, lateral.
Fig. 15. Vulvar region, lateral.
Fig. 16. Vulvar region, lateral.

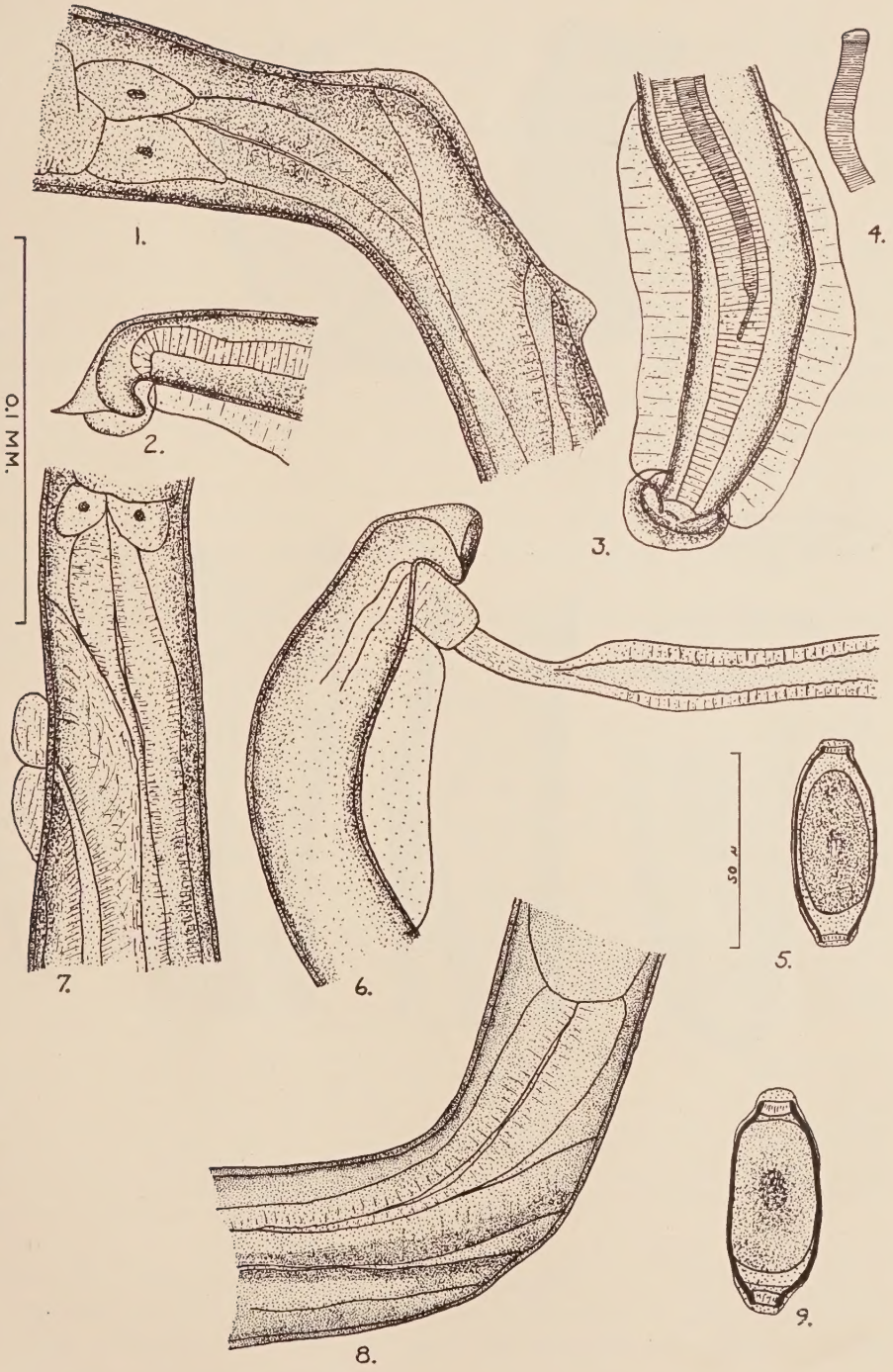
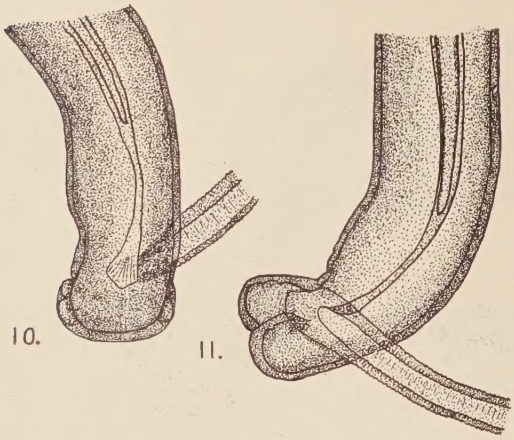


PLATE I



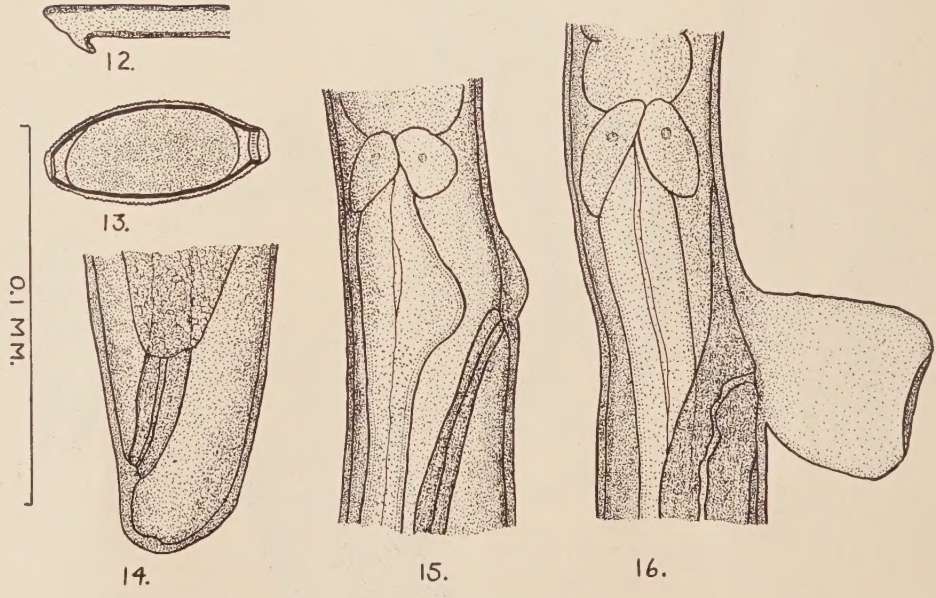


PLATE II

STUDIES ON NORTH AMERICAN HELMINTHS OF THE GENUS *CAPILLARIA* ZEDER, 1800 (NEMATODA): II. ADDITIONAL CAPILLARIDS FROM MAMMALS WITH KEYS TO THE NORTH AMERICAN MAMMALIAN SPECIES*

CLARK P. READ

As previously remarked by the writer in Part I of this study the worms of the genus *Capillaria* Zeder, 1800, do not show a marked degree of host specificity. Further evidence in favor of this view is afforded by the fact that *C. americana* n. sp., described in this paper, has been found in four different species of rodents. *C. muris-sylvatici* (Diesing, 1851) evidently also occurs in a variety of rodent hosts. Elton, Ford, and Baker (1931) in a study of the parasites of various rodent populations in England failed to find this worm in *Microtus* although these workers found it in other rodents. Conversely, the writer has found it only in material from *Microtus* in North America although large collections of parasites from a considerable number of different native rodents have been available for study. It seems probable that food habits are the chief factor determining the acquisition of infections with this species by rodents.

Description or discussion of five species of *Capillaria* from North American mammals follows:

Capillaria muris-sylvatici (Diesing, 1851)
(Plate II, Figs. 10–13)

Worms of this species were recovered from the small intestine of the vole, *Microtus p. pennsylvanicus,* examined at Madison, Wisconsin. No previous record from this host nor from any North American host has been found in a search of the literature. Since the worms show some features not recorded by other workers, a description of this material seems warranted.

Description: Lateral bacillary lines visible. Mouth simple. Cuticle striated transversely. *Female:* 13.4 to 15.1 mm long; maximum width in posterior region 70 to 75 μ. Termination of esophagus 3 mm from anterior end. Vulva just posterior to termination of esophagus, 3 to 3.2 mm from anterior end, with cuticular vulvar flap projecting from region of anterior labium. A discoid cuticular projection is located anterior to vulvar flap at level of esophageal termination. Vulva divides body 1 : 3.5 to 1 : 3.7. Ova 47 to 60 μ long by 24 to 26 μ wide; inner shell bends to form collar at each end; outer shell smooth. Anus slightly subterminal.

Male: 9.1 to 10.8 mm long; maximum width 45 to 47 μ. Termination of esophagus 2.7 to 2.9 mm from anterior end. Lateral caudal alae present, about 70 μ long. Spicule 204 to 225 μ long by 11 to 13 μ wide. Spicule sheath without spines or transverse striations. Everted spicule sheath divided into four portions: Distal portion delicate, membranous, usually wrinkled; subdistal portion bulblike, muscular; proximal portion bulblike with rugose markings on lateral aspect; proximal and subdistal portions joined by short wrinkled tubular projection of proximal bulb. Cuticular bursa supported by pair of bifid papillae. Cloaca subterminal. Termination of esophagus divides body about 1 : 2.7.

According to Teixeira de Freitas and Lent (1936) this species was studied by Kalantarian (1924), whose work has not been accessible to the writer. Kalantarian renamed the species *C. halli* which was reduced to synonymy by Teixeira de Freitas and Lent. These workers, following Kalantarian, described a toothpick-like papilla

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* Contribution from the Biological Laboratories of The Rice Institute, Houston, Texas, and Tulane University, New Orleans, Louisiana.

projecting from the vaginal aperture, and such a structure is shown in their figure taken from Kalantarian. The writer has found that if the female worms are viewed from a dorso-lateral angle the cuticular flap appears as such a papilla. Considerable manipulation of specimens is necessary to obtain the true picture of the vulvar anatomy. The male worms agree essentially with the description given by Teixeira de Freitas and Lent, except that they apparently do not have a pair of submedian ventral bursal papillae. The writer at first referred the material at hand to a new species, but the correspondence of the prevulvar cuticular projection and vulvar flap in the female and the bifurcate caudal papillae and caudal alae in the male to the described features of *C. muris-sylvatici* seem to warrant the reference of these worms to that species.

Although it is obvious that the everted spicule sheath of capillarids may be highly variable in form, the writer has found that this structure appears essentially as described above in fifteen male worms whose sheaths were everted. This seems to be a rather constant feature.

Capillaria bovis (Schnyder, 1906) Ransom, 1911

This worm was described very fragmentarily by Schnyder (1906) from *Bos taurus* in Europe. In 1911 Ransom described a worm, *Capillaria longipes*, from North American prong-horned antelope and sheep. Ransom's adequately described worm differed from the poorly described European form in hostal and geographical distribution. Restudy by Petrow and Orlow (1930) of *C. bovis* from *Bos taurus* in Europe led Teixeira de Freitas and Lent (1936) to regard *C. longipes* as a synonym of *C. bovis*. The writer agrees with this view. Teixeira de Freitas and Lent (1936) suggested that restudy of the type material of *C. brevipes* Ransom, 1911, from North American sheep, might show this worm to be cospecific with *C. bovis*.

Through the courtesy of Dr. E. W. Price, the writer has been able to examine the types of *C. longipes* Ransom, 1911, and *C. brevipes*, Ransom, 1911, which are deposited in the U. S. National Museum. The specimens of *C. longipes* conform closely with the description of *C. bovis* as given by Teixeira de Freitas and Lent. The type specimens of *C. brevipes* correspond with Ransom's description in all respects except one. Ransom noted that the shell of the egg of *C. brevipes* was 3 to 4 μ thick, while that of *C. longipes* was 1.5 to 2 μ thick. Ransom's figures of the eggs of the two species also shows this difference in shell thickness. The writer has been unable to find any difference in the thickness of the shell nor has any other difference in the eggs been noted. It would seem that the main differences between the two species are differences in size. The lengths of the male and female of *C. bovis*, following the description of Teixeira de Freitas and Lent, are 11 to 13 mm. and 18 to 25 mm. respectively; the lengths of the male and female of *C. brevipes* are 8 to 9 mm. and 12 mm. respectively. In the light of these observations it would seem that the two species, *C. bovis* and *C. brevipes*, must be recognized.

Dikmans (1930) has reported *C. brevipes* from cattle in the United States. It would be of some interest to compare material from cattle with that from sheep. The similarity of structure of *C. bovis* and *C. brevipes* can leave little doubt that the two species are closely related and future study may reveal that the limits of variation of *C. bovis* are broad enough to encompass the form now recognized as a second species.

Capillaria michiganensis n. sp.

(Plate I, Figs. 3-4)

Specific diagnosis: Body finely striated transversely. Bacillary lines absent. Mouth simple.

Female: 17.5 to 20.9 mm. long; width just posterior to vulva 45 to 48 μ; maximum width 62 to 70 μ. 33 to 35 paraesophageal nuclei. Vulva just posterior to termination of esophagus and 6.7 to 9.0 mm. from anterior end. Anterior labium salient, forming flap over vulva. Vulva divides body 1 : 1.0 to 1 :1.6. Eggs 53 to 56 μ by 28 to 30 μ; inner shell bends to form collar around plug at each end; outer shell smooth.

Anus subterminal.

Male: Unknown.

Host: Ondatra zibethica.

Habitat: Small intestine.

Locality: Monroe County, Michigan.

Type: U. S. National Museum, Helminth. Coll. No. 46366.

Female worms taken from the small intestine of Michigan muskrats are referred to this species. These worms had been examined by Dr. J. D. Tiner and tentatively referred to *Capillaria ransomia* Barker and Noll, 1915, a species previously recorded from muskrats. However, the worms examined by the writer differ rather markedly from the description of *C. ransomia* given by Barker (1915). Barker stated that the vulva is in the anterior fourth of the body in *C. ransomia;* in the worms examined by the writer the vulva is in the second fourth. Barker gave the egg measurements of *C. ransomia* as 50 by 20 μ, whereas the eggs from the worms examined in the present study are 53 to 56 μ by 28 to 30 μ. So far as Barker's description indicates, *C. ransomia* lacks a vulvar flap. This character serves to separate *C. michiganensis* from all other species in North American mammals, except *C. muris-sylvatici.* Hall (1916) has already pointed out that the indicated magnifications in Barker's figures do not agree with the measurements in the text. The error seems to lie in the scale appended to the figures. Specimens from the muskrat identified as *C. ransomia* were loaned to the writer by Dr. E. W. Price. They were female worms collected in Connecticut and are identical with those from Michigan muskrats.

A single male worm recovered from the present series of muskrats has not been accessible to the writer. Tiner (personal communication) examined this worm and stated that the spicule was 1.53 mm. long. Tiner also noted the discrepancy between the position of the vulva in the female worms from this series and those described by Barker. The single male examined by Tiner possibly was *C. ransomia,* since Barker gave the spicule length of this species as 1.36 mm.

It should be mentioned that nine of one hundred muskrats were found to be infected with *Capillaria.* However, the writer examined material from only three of these hosts.

Capillaria chandleri n. sp.

(Plate I, Figs. 1-2)

Specific diagnosis: Body finely striated transversely. Bacillary lines absent. Mouth simple.

Female: 15.3 mm. long; 46 μ wide just posterior to vulva; maximum width 72 μ. Body with marked prevulvar swelling posterior to which is prevulvar constriction. Vulva situated 3.7 mm. from anterior extremity; labia markedly salient. Ova 57 to 58 μ long by 28 μ wide, outer shell smooth; inner shell thin and forming collar at either end.

Anus terminal. Vulva divides body 1 : 3.1.

Male: Unknown.

Host: Citellus franklini.

Habitat: Small intestine.

Locality: Madison, Wisconsin.

Type: U. S. National Museum, Helminth. Coll. No. 46355.

Two females of this species, one of which was incomplete, were recovered from one of ten Franklin ground squirrels examined. *C. chandleri* differs from all other species of *Capillaria* in North America in the peculiar prevulvar swelling of the body. This was seen in both specimens.

Capillaria americana n. sp.
(Plate I, Figs. 5-7).

Specific diagnosis: Body transversely striated. Thin lateral bacillary lines visible in some specimens. Four minute papillae around mouth.

Female: 23 to 28.4 mm. long; 106 to 118 μ wide just posterior to vulva; maximum width 136 to 144 μ. 36 to 39 paraesophageal nuclei. Vulva slightly posterior to termination of esophagus, 6.8 to 7.5 mm. from anterior extremity; labia slightly salient; heavily muscular ovejector present. Body cavity filled by gravid uterus. Ova 46 to 52 μ by 23 to 27 μ; outer shell lightly striated; inner shell forming slight collar for opercular plug at each end. Anus slightly subterminal. Vulva divides body 1 : 2.4 to 1 : 2.7.

Male: 12.2 to 15.4 mm. long; maximum width 84 to 103 μ. 41 to 45 paraesophageal nuclei. Termination of esophagus 5.4 to 6.5 mm. from anterior end. Spicule sheath smooth. Spicule stout, transversely striated, 209 to 258 μ long by 11 to 14 μ wide. Cauda terminated by two poorly developed lobes; single minute papilla on ventrum of each lobe. Bursa lacking. Cloacal opening slightly subterminal. Termination of esophagus divides body 1 : 1.0 to 1 : 1.7.

Hosts: Glaucomys volans volans (type). *Sciurus carolinensis leucotis, Peromyscus maniculatus bairdii, Peromyscus leucopus noveboracensis.*

Habitat: Small intestine.

Locality: McHenry, Illinois (type), Marysville, Ohio, and Madison, Wisconsin.

Type: U. S. National Museum, Helminth. Coll. No. 46368.

Worms of this species were recovered from the small intestine of seven flying squirrels at McHenry, Illinois, and Marysville, Ohio, from one of forty-six prairie white-footed mice at Madison, Wisconsin, from two of twenty-seven gray squirrels at Madison, Wisconsin, and from one of one hundred and twenty-nine northern white-footed mice at Madison, Wisconsin. The distribution of this worm in squirrels under the designation *"Capillaria sp."* is discussed by Rausch and Tiner (1948).

The posterior end of the male of *C. americana* shows some similarity to that of males of *C. aerophila* from the lungs of carnivores, but the two species are quite different in other respects. The stout spicule and the cauda of the male are quite adequate for differentiation from other mammalian species of *Capillaria.*

KEYS TO THE SPECIES OF *Capillaria* PARASITIC IN NORTH AMERICAN MAMMALS

Females

1. *a.* From alimentary canal 2
   b. From location other than alimentary canal 4
2. *a.* Worms about 75 mm. long. Tail with two conical projections on ventral surface ..... *linearis* (Leidy, 1856).
   b. Worms considerably less than 75 mm. long 3
3. *a.* Vulva in anterior fourth of body 7
   b. Vulva not in anterior fourth of body 10
4. *a.* From skin of primates. Vulva divides body about 1 : 5. Ova 67 to 70 by 40 to 42 μ ..... *cutanea* (Swift, Boots, and Miller, 1922).
   b. From viscera 5
5. *a.* From trachea, bronchi, and lungs of carnivores. Vulva at level of termination of esophagus, without appendage ..... *aerophila* (Creplin, 1839).

b. From liver. Vulva with tubular appendage .... *hepatica* (Bancroft, 1893).
c. From urinary bladder of carnivores 6
6. *a.* Vulva with campanuliform appendage. Posterior end obtuse with anus terminal ..... *plica* (Rudolphi, 1819).
b. Vulva with slightly salient labia. Posterior end with three slight lobes surrounding terminal anus ..... *felis-cati* (Diesing, 1851).
7. *a.* Vulva with anterior cuticular flap and a discoid cuticular prevulvar projection. Ova 47 to 60 by 24 to 26 μ ..... *C. muris-sylvatici* (Diesing, 1851).
b. Vulvar anatomy other than as in *a* 8
8. *a.* Body with marked prevulvar swelling. Labia salient. Ova 57 to 58 by 27 to 28 μ. From Sciuridae ..... *chandleri* n. sp.
b. Body without marked prevulvar swelling 9
9. *a.* Anus terminal. Ova 64 to 72 by 28 to 32 μ. From carnivores. *putorii* (Rudolphi, 1819).
b. Anus subterminal. Ova 50 by 20 μ. From *Ondatra zibethica*. *ransomia* Barker and Noll, 1915.
10. *a.* Vulva with anterior cuticular flap. Ova 53 to 56 by 28 to 30 μ. From *Ondatra zibethica* ..... *michiganensis* n. sp.
b. Vulvar anatomy other than as in *a*, with or without appendage 11
11. *a.* Vulva with tubular appendage 12
b. Vulva without tubular appendage. Labia slightly salient or not salient .. 13
12. *a.* Worms 21 to 22 mm. long. Vulva divides body about 1 : 2.2. Ova 47 to 50 by 31 to 32 μ. From Vespertilionidae ..... *palmata* Chandler, 1938.
b. Worms 5.2 to 7.3 mm. long. Vulva divides body 1 : 1.6 to 1 : 1.9. Ova 61 by 27 μ. From Soricidae ..... *rauschi* n. sp.
13. *a.* Vulva with salient or slightly salient labia 14
b. Vulva without salient labia 16
14. *a.* Vulva with slightly salient labia. Heavily muscular ovejector present. Body filled by gravid uterus ..... *americana* n. sp.
b. Vulva with markedly salient labia. Body not filled by gravid uterus 15
15. *a.* Worms 18 to 25 mm. long. Ova 45 to 52 by 21 to 30 μ. From Ungulata. *bovis* (Schnyder, 1906).
b. Worms 10.5 to 15.5 mm. long. Ova 50 to 57 by 25 to 27 μ. From Sciuridae. *tamias-striati* n. sp.
16. *a.* Anus terminal. Ova 50 by 25 μ. From Ungulata .. *brevipes* Ransom, 1911.
b. Anus subterminal. Ova 52 to 60 by 24 to 30 μ. From Carnivora. *mustelorum* Cameron and Parnell, 1932.

Males

1. *a.* From alimentary canal 2
b. From location other than alimentary canal 11
2. *a.* Worms about 36 mm. long. Cloaca considerable distance from posterior end. *linearis* (Leidy, 1856).
b. Worms less than 20 mm. long. Cloaca terminal or subterminal 3
3. *a.* With lateral caudal alae 4
b. Without lateral caudal alae 9

4. *a.* Spicule sheath smooth. Membranous bursa supported by two papillae; distal portion of each papilla turned cephalad at right angles to proximal portion. Found in ruminants 5
   b. Spicule sheath transversely striated. Membranous bursa supported by papillae which are different from *a.* Not found in ruminants 6
5. *a.* Spicule 1000 to 1200 μ long. Termination of esophagus divides body 1 : 1.7. *bovis* (Schnyder, 1906).
   b. Spicule 900 μ long. Termination of esophagus divides body 1 : 1.2. *brevipes* Ransom, 1911.
6. *a.* Termination of esophagus in anterior third of body. Spicule less than 225 μ long 14
   b. Termination of esophagus not in anterior third of body. Spicule more than 225 μ long 7
7. *a.* Tips of caudal lobes bifurcate. Lateral bursal "rays" present. From Vespertilionidae ..... *palmata* Chandler, 1938.
   b. Tips of caudal lobes not bifurcate. Lateral bursal "rays" not present 8
8. *a.* Spicule 490 to 502 μ long. Lateral caudal alae sometimes ribbed. *tamias-striati* n. sp.
   b. Spicule 290 to 406 μ long. Lateral caudal alae not ribbed. *mustelorum* Cameron and Parnell, 1932.
9. *a.* Cauda with two stout papillae supporting bursa 10
   b. Cauda with two poorly defined lobes. Bursa lacking. Spicule 209 to 258 μ long by 11 to 14 μ wide ..... *americana* n. sp.
10. *a.* Worms about 4.8 mm. long. Spicule 395 μ long. From Soricidae. *rauschi* n. sp.
    b. Worms about 19.6 mm. long. Spicule 1360 μ long. From *Ondatra zibethica.* *ransomia* Barker and Noll, 1915.
11. *a.* From urinary bladder of carnivores. Spicule sheath without spines 12
    b. From viscus other than urinary bladder. Spicule sheath finely spiny ... 13
12. *a.* Spicule 4 mm. long ..... *plica* (Rudolphi, 1819).
    b. Spicule 2.5 mm. long ..... *felis-cati* (Diesing, 1851).
13. *a.* From trachea, bronchi, and lungs of carnivores. Spicule very thin, usually not visible ..... *aerophila* (Creplin, 1839).
    b. From liver. Spicule well developed ..... *hepatica* (Bancroft, 1893).
14. *a.* Spicule 192 to 225 μ long. Caudal papillae bifurcate. *muris-sylvatici* (Diesing, 1851).
    b. Spicule 149 to 168 μ long. Caudal papillae stout, not bifurcate. *putorii* (Rudolphi, 1819).

Not included in the key to the males are *C. michiganensis, C. chandleri,* and *C. cutanea;* only the females of these worms are known.

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* References not listed may be found in Part I of this study, appearing earlier in this journal.

Plate I

Capillaria chandleri

Fig. 1. Egg.
Fig. 2. Vulvar region, lateral.

Capillaria michiganensis

Fig. 3. Vulvar region, lateral.
Fig. 4. Egg.

Capillaria americana

Fig. 5. Vulvar region, lateral.
Fig. 6. Spicule.
Fig. 7. Egg.
Fig. 8. Cauda of male, ventral.
Fig. 9. Anterior end.

Plate II

Capillaria muris-sylvatici

Fig. 10. Cauda of male, spicule sheath everted, lateral.
Fig. 11. Cauda of male spicule sheath retracted, ventral.
Fig. 12. Cauda of male, spicule sheath everted, ventral.
Fig. 13. Vulvar region, lateral.

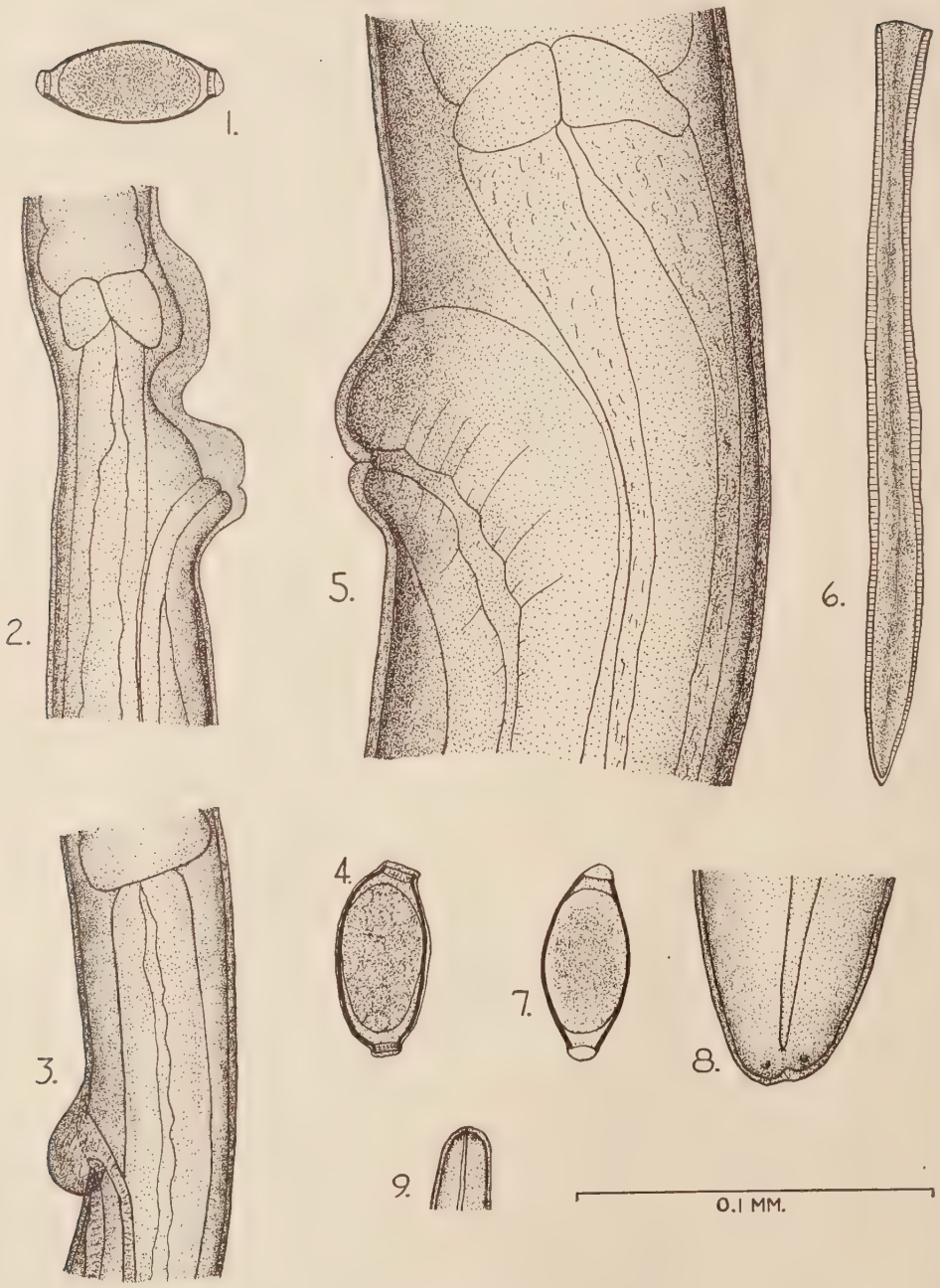

PLATE I

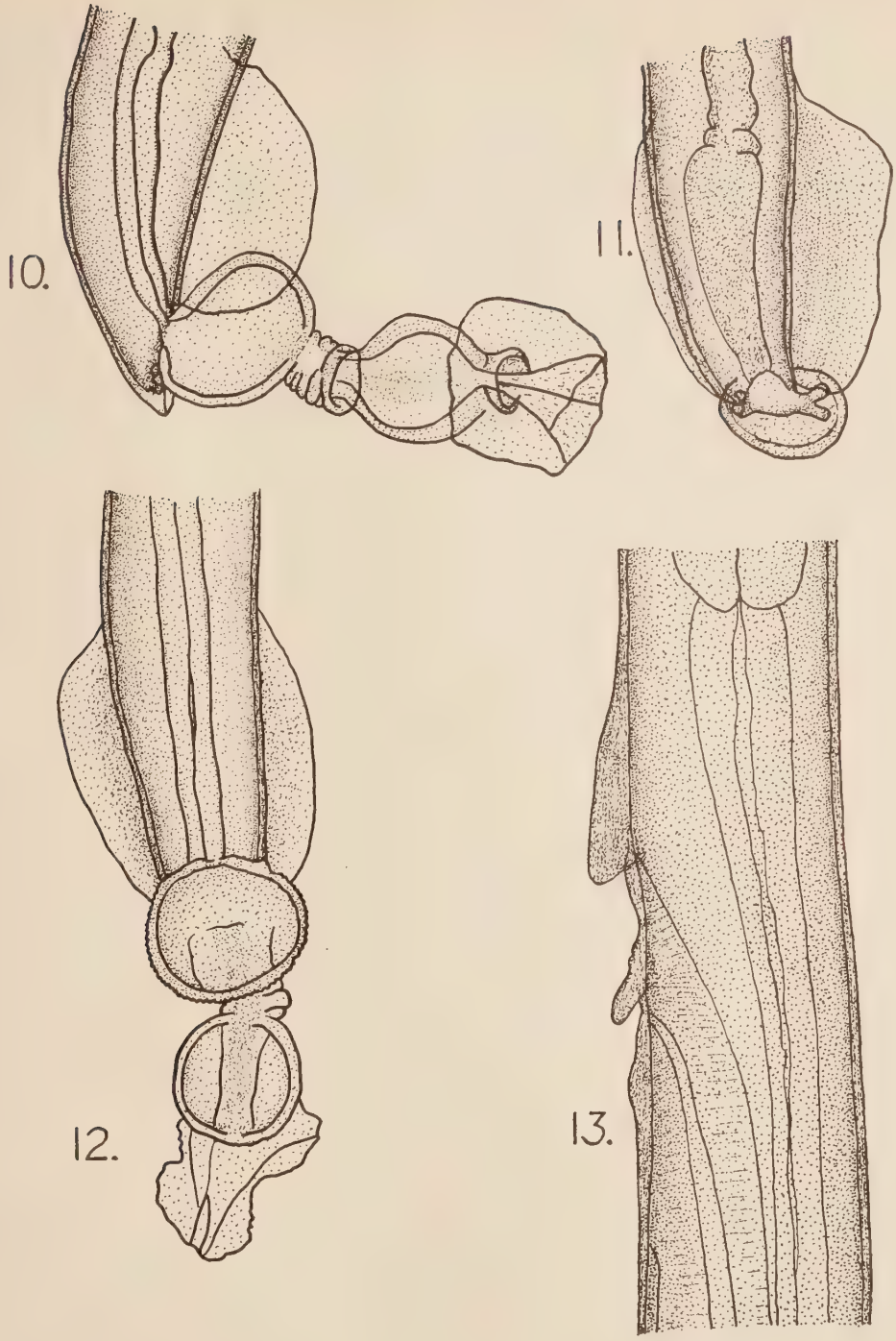

PLATE II

STUDIES ON NORTH AMERICAN HELMINTHS OF THE GENUS *CAPILLARIA* ZEDER, 1800 (NEMATODA): III. CAPILLARIDS FROM THE LOWER DIGESTIVE TRACT OF NORTH AMERICAN BIRDS *

CLARK P. READ

The present portion of this study is limited to those species of *Capillaria* inhabiting the lower digestive tract of American birds. None of the writer's specimens from the upper digestive tract of birds revealed features which were not adequately dealt with in the excellent work of Teixeira de Freitas and Lins de Almeida (1935) and Cram (1936). Most of the material examined was collected by Dr. Robert Rausch, of the Department of Veterinary Science, University of Wisconsin, who kindly made the material available for study. The writer wishes to express gratitude to Dr. E. W. Price and Mr. Allen McIntosh for their patient attention and advice regarding some of the nomenclatorial difficulties in which the writer found himself entangled during this and other portions of this study.

A review of the literature reveals that at least nine species of *Capillaria* have been reported previously from the lower digestive tract of birds on this continent. In the present study four species, two of which are new, are added to the known avian parasitic fauna of North America and some attempt at the clarification of the nomenclature of two other species is made. In addition, keys to the North American species have been devised.

Capillaria caudinflata (Molin, 1858)

Two female worms of this species were recovered from the small intestine of a starling, *Sturnus vulgaris,* of twenty-five examined at East Lansing, Michigan. Several chickens examined by the writer at Houston, Texas also harbored this parasite. Since the material conforms closely with the descriptions of *Trichosoma longicolle* of Shipley (1909) and *Capillaria longicollis* of Morgan (1932), no description of the material at hand seems necessary.

Cram (1925) reported this helminth for the first time from North American birds as *C. meleagris-gallapavo* from the turkey. Morehouse (1939) reported it from chickens in Iowa, Minnesota, Ohio, Illinois, Wisconsin, Pennsylvania, Missouri, Kansas, Indiana, and Michigan. Todd (1946) reported it from chickens in Tennessee. Morehouse (1944) described the life cycle and reported the turkey and the English sparrow, *Passer domesticus,* as experimental hosts.

Cannon (1939) reported *C. columbae* var. *sturni* from *Sturnus vulgaris* in Quebec. However, the female of the species described under the name *C. columbae* by other workers does not possess a vulvar appendage; Cannon stated that his specimens had at the vulva "an external membranous protrusion." It seems probable to the writer that Cannon's specimens should be referred tentatively to *C. caudinflata* which possesses such an appendage.

Madsen (1945), in an extensive study of capillarids from gallinaceous and

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anatine birds, concluded that the correct name for this species is "*Capillaria longicollis* (Mehlis 1831)." Madsen stated, "As *Capillaria longicollis* in its modern delimitation occurs only in the small intestine, the species very fragmentarily described by Rudolphi under the above mentioned name does unfortunately not belong to this species. But as Mehlis (1831) has found in the small intestine of the pheasant a species which he named *Capillaria longicollis* and the name in the present delimitation has been current for a number of years, it seems reasonable to retain it, however with Mehlis as author."

The writer cannot agree with this opinion of Madsen. In the first place it should be said that Madsen attributes the name to a man who did not make it. If Mehlis' *Trichosomum longicolle* is not the same species as *Trichosoma longicolle* Rudolphi, 1819, Mehlis misidentified the specimens before him. The worms described by Madsen and other workers from the small intestine are probably not *C. longicollis* (Rudolphi, 1819), described from the large intestine of the pheasant, but a distinct species. As Morehouse (1944) pointed out, the first available name applied unquestionably to this zoological entity from the small intestine was *Calodium caudinflatum* Molin, 1858. Thus, the correct name for this worm is *Capillaria caudinflata* (Molin, 1858). *C. collaris* Linstow, 1873, or *C. cadovulvata* Madsen, 1945, from the cecum of the pheasant and the quail may be the same species as *Trichosoma longicolle* Rudolphi.

Capillaria tridens (Dujardin, 1845)
(Plate I, Figs. 1–2)

A single male worm of this species was taken from the small intestine of one Red-winged Blackbird of forty-four examined at Prairie du Sac, Wisconsin. A description follows:
Diagnosis: Body transversely striated. Bacillary lines absent. Mouth simple.

Male: 13.9 mm. long; maximum width 49 μ. Termination of esophagus 6.2 mm. from anterior end. Spicule 1.275 mm. long, 18 μ wide near proximal end; spicule sheath 1.840 mm. long by 24 μ wide, beset with spines. Cauda broadly terminated by three stout lobes. Slightly developed membranous bursa present. Cloaca subterminal. Termination of esophagus divides body 1 : 1.3.

Host: Agelaius phoeniceus.

Habitat: Small intestine.

Locality: Prairie du Sac, Wisconsin.

This worm has not previously been reported from North America and has been reported only from the type host, *Luscinia luscinia,* the thrush-nightingale, in other parts of the world. The species is easily recognized by the broad trilobed tail of the male.

Capillaria falconis-nisi (Diesing, 1851)
(Plate I, Figs. 3–7)

Description: Body finely striated transversely. Lateral bacillary lines one-fourth of body diameter or not visible. Mouth simple.

Female: 16.4 to 29.4 mm. long; width just posterior to vulva 60 to 69 μ; maximum width 87 to 100 μ. Vulva transverse slit, 5.0 to 7.7 mm. from anterior end, just posterior to termination of esophagus; labia slightly salient. Ova 65 to 72 μ by 30 to 34 μ; inner shell bent at poles to form collar around plug; outer shell finely reticulated. Anus subterminal. Vulva divides body 1 : 2.0 to 1 : 2.7.

Male: 9.6 to 14.8 mm. long; maximum width 54 to 80 μ. Termination of esophagus 3.3 to 6.9 mm. from anterior end. Spicule lightly striated transversely, 750 to 1250 μ long by 12 to 14 μ wide; tip blunt. Spicule sheath finely striated transversely, 1.20 to 1.35 mm. long when completely everted. Cauda with two stout lobes supporting a slightly developed cuticular bursa; single minute papilla sometimes visible on ventral surface of each lobe. Cloacal opening subterminal. Termination of esophagus divides body 1 : 0.9 to 1 : 1.9.

Hosts: Asio wilsonianus, Cryptoglaux acadica, Bubo virginianus virginianus, Buteo borealis.

Habitat: Small intestine.

Locality: Wisconsin.

Worms of this species were taken from a single Long-eared Owl at Horicon Marsh, Wisconsin, from a single Saw-whet Owl at Poynette, Wisconsin, from eight of fifty-two Great Horned Owls at Poynette, Wisconsin, and from three of forty-two Red-tailed Hawks at Poynette, Wisconsin. This worm has not been reported previously from North America.

The male of this species was described as *Trichosomum contortum* from *Accipiter nisus* in Europe by Dujardin (1843). The error was corrected and the species named by Diesing (1851). It is interesting to note that the female of *C. falconum* (Rudolphi, 1819), described from the same host, is indistinguishable, by extant descriptions, from the female of *falconis-nisi.* Only the female of *falconum* is described; the male is mentioned as having a finely spinous spicule sheath. The inadequately described *C. striata* (v. Linstow, 1879) also differs from *falconis-nisi* in the possession of a spiny spicule sheath. Madsen (1945) regards *C. striata* as a probable synonym of *C. falconum.*

It has been found by the writer in examining male capillarids that unless the spicule sheath is everted, it is sometimes almost impossible to determine whether or not the sheath is finely spined. It is possible that the three species, *falconum, striata,* and *falconis-nisi,* are identical. It should be pointed out that the presence or absence of fine spines on an inverted spicule sheath is sometimes difficult to determine in other species of this genus.

A pertinent example of a mistake of this kind may be seen in the case of *C. collaris* (Linstow, 1873), described as having a finely spined spicule sheath. Railliet (1895) described a capillarid, *Trichosomum retusa,* which differed from *C. collaris* in lacking spines on the spicule sheath. Later investigators found worms which were obviously *C. retusa,* but which had fine spines on the spicule sheath. Teixeira de Freitas and Almeida (1935) reached the conclusion that *C. retusa* (Railliet, 1895) is a synonym of *C. collaris* (Linstow, 1873). Similar examples will probably be uncovered in this genus by future workers.

Capillaria obsignata Madsen, 1945

Worms of this species were collected from the small intestine of three of forty-nine robins, *Turdus migratorius,* taken at Columbus, Ohio, and Madison, Wisconsin, and from the domestic pigeon, *Columba livia domestica,* at New Orleans, Louisiana.

Other workers have reported and described this worm from the small intestine of pigeons in many parts of the world, under the name *Capillaria columbae* (Rud.). According to Wehr (1939), this worm has been taken from the pigeon in Maryland, New Jersey, South Carolina, and District of Columbia. Miller (1937) has reported it from this host in Quebec. It has been reported from the chicken in at least twelve states east of the Rocky Mountains by Graybill (1924), Levine (1938), Morehouse (1939), and Todd (1946). The turkey was reported as a host in New Jersey by Graybill (1924). According to Levine (1938) and Morehouse (1944), Cram (1931) experimentally infected a quail with this helminth. Levine (1938) and Wehr (1937, 1939) described the life cycle. Madsen (1945) has pointed out that *Trichosoma columbae* Rud., 1819 was proposed for specimens from the large intestine of the pigeon, and that Rudolphi's specimens were described under the name *Calodium tenue* by Dujardin (1845), who reported that the females possessed a projecting vulvar appendage, a character which is lacking in the species commonly

found in the small intestine of this host. Further, Madsen stated that the first recognizable description of the capillarid from the small intestine of pigeons was that given under the name *C. dujardini* by Travassos (1915). This is true. Unfortunately, however, Travassis in 1914 proposed *Capillaria dujardini n. nom.* for *Calodium tenue* Dujardin, 1845, the name *tenue* being preoccupied by page preference by *Trichosomum tenue* Dujardin, 1845. Dujardin (1845) in his description of *Calodium tenue* places *"Trich. columbae, Rudolphi—"* as a synonym. It would appear then that the name *C. dujardini* Travassos, 1914, must be applied to a form from the large intestine with a projecting vulvar appendage.

Travassos (1915) in describing the female of *C. dujardini* Trav., 1914 states "vulva circular, de labios lijeiramente salientes." And he gives as the habitat "Intestina delgado de *Columba livia* L. e *Columba livia dom.* L." It would appear that Travassos in 1915 misdetermined his material, since it is evident that he was not dealing with the same species that Dujardin had described from the pigeon, and yet it was Dujardin's species to which he (Tavassos) gave this name in 1914. This being the case, a name must be selected for the species in which the vulva is circular and slightly projecting and which lives in the small intestine of the pigeon.

Madsen (1945) has referred *C. columbae* of Graybill (1924) to a new species, *C. obsignata.* Madsen evidently came to this decision solely on the basis of Graybill's description and apparently was influenced to regard Graybill's specimens as differing specifically from *C. columbae* of other authors because of the structure of the eggs as figured by Graybill. The writer has been unable to locate the material from chickens and turkeys which Graybill utilized in preparing his description; however, Dr. E. W. Price has kindly made available for study material from the pigeon at Washington, D. C. This material (U.S.N.M. Helm. Coll. No. 15144) was identified by Graybill as *C. columbae,* and it seems that this is the material mentioned by Graybill as having been compared with his worms from chickens and turkeys. This material is specifically identical with the writer's specimens from the pigeon. The eggs do not possess a collar as described and figured by Graybill. It seems that Graybill made a misinterpretation of the egg structure in his material, since his description agrees in other respects with descriptions of *C. columbae* of other workers. According to Mr. Allen McIntosh (personal communication) of the Bureau of Animal Industry, the material on slide number 15144 may be considered as a part of the type material of *C. obsignata.*

It is, therefore, concluded that there are two capillarids from the pigeon with the synoymy in part as follows:

1. From large intestine:
   Capillaria columbae (Rudolphi, 1819).
   Syn. *Calodium tenue* Dujardin, 1845.
   Trichosomum (Calodium) tenuissimum Diesing, 1851.
   Capillaria dujardini Travassos, 1914.
2. From small intestine:
   Capillaria obsignata Madsen, 1945.

Capillaria quiscali n. sp.
(Plate II, Figs. 9–13)

Specific diagnosis: Bacillary lines absent. Cuticle transversely striated. Mouth simple.

Female: 8.5 to 10.3 mm. long; 65 to 70 μ wide just posterior to vulva; maximum width 84 to 102 μ. Vulva bearing a funnel-shaped cuticular appendage, located 2.9 to 4.5 mm. from anterior extremity just posterior to termination of esophagus. Ovejector well developed, heavily muscular and salient, S-shaped when retracted. Ova 57 to 66 μ long by 25 to 30 μ wide; inner shell forms collar around plug at each end; outer shell roughly mammillated. Anus subterminal. Vulva divides body 1 : 1.5 to 1 : 2.0.

Male: 8.2 mm. long; maximum width 72 μ. Termination of esophagus 3.9 mm. from anterior extremity. Spicule smooth with blunt tip, 962 μ long; Spicule sheath transversely striated, 1.1 mm. long. Cauda provided with lateral cuticular alae continuous with membranous bursa. Bursa supported by two stout papillae; each papilla split into blunt dorsal and ventral rami. Cloaca subterminal, flanked on either side by small papilla. Termination of esophagus divides body 1 : 1.3.

Host: Quiscalus quiscala aeneus.
Habitat: Small intestine.
Locality: Madison, Wisconsin.
Type: U. S. National Museum, Helminth. Coll. No. 46354.

Worms of this species were recovered from two Bronzed Grackles of eighteen examined. This species bears some resemblance to *C. caudinflata* (Molin, 1858), differing from it in the larger size and rough outer shell of the eggs, the fusion, in the male, of the caudal alae with the membranous bursa, and the somewhat smaller size of the worms.

Capillaria freitasi n. sp.
(Plate II, Figs. 14–15)

Specific diagnosis: Body smooth. Bacillary lines absent. Mouth simple.
Female: Unknown.
Male: 11.5 mm. long; maximum width 46 μ. Termination of esophagus 5.9 mm. from anterior end. Spicule smooth with blunt tip, 900 μ long. Spicule sheath smooth, 1.825 mm. long by 13 μ wide when completely everted. Cauda terminated by two stout lobes supporting membranous bursa. Cloaca slightly subterminal. Termination of esophagus divides body 1 : 0.9.
Host: Passarella iliaca iliaca.
Habitat: Intestine.
Locality: Madison, Wisconsin.
Type: U. S. Natl. Mus., Helminth. Coll. No. 46356.

A single male of this species was recovered from one of nine fox sparrows examined at Madison. *C. freitasi* bears some resemblance to *C. falconis-nisi* (Diesing, 1851) reported elsewhere in this paper from owls and hawks. However, the spicule and spicule sheath of this species are definitely smooth in contrast to the striated structure of these organs observed in *falconis-nisi.*

This species is named in honor of Dr. J. F. Teixeira de Freitas who has made many valuable contributions to the knowledge of the helminths of this genus.

Capillaria sp.
(Plate II, Fig. 8)

Single immature female worms of this genus were recovered from the small intestine of two Scarlet Tanagers of six examined at Madison and Shawano, Wisconsin. It has not been possible to refer these worms to any known species.

Description: Body transversely striated. Lateral bacillary lines one-fourth diameter of body; slight dorsal and ventral bacillary lines present. Mouth simple.

Female: Immature. 11.5 and 20.2 mm. long. Width just posterior to vulva 64 and 76 μ; maximum width 76 and 98 μ. Forty-eight paraesophageal nuclei. Vulva 4.6 and 7.8 mm. from anterior end, with tubular cuticular appendage. Eggs absent. Anus subterminal. Vulva divides body 1 : 1.5 and 1 : 1.6.

Male: Unknown.
Host: Piranga erythromelas.
Habitat: Small intestine.
Locality: Wisconsin.

The cuticular vulvar appendage resembles that seen in some specimens of *C. caudinflata;* however, females of that species would be expected to have reached maturity before attaining the size of the worms described above. It is possible that *caudinflata* is incapable of reaching maturity in this host, but, in view of the fact that mature female *caudinflata* have been reported from such diverse hosts as chicken, partridge, pheasant, grouse, pigeon, starling, and English sparrow, this would seem doubtful.

KEYS TO THE SPECIES OF *Capillaria* PARASITIC IN THE LOWER DIGESTIVE TRACT OF NORTH AMERICAN BIRDS.

Females

1. *a.* Vulva with well developed appendage 2
   b. Vulva without appendage 4
2. *a.* Anus terminal, Ova 43 μ long ..... *picorum* (Rudolphi, 1819)
   b. Anus subterminal 3
3. *a.* Ova 47 to 55 μ long; outer shell smooth. A prevulvar notch present. *caudinflata* (Molin, 1858).
   b. Ova 57 to 66 μ long; outer shell roughly mammillated. No prevulvar notch. *quiscali* n. sp.
4. *a.* Body with pre- and post-vulvar cuticular bosses. *bursata* Teixeira de Freitas and Almeida, 1934.
   b. Body without pre- and post-vulvar cuticular bosses 5
5. *a.* Inner shell of egg bent to form a collar 6
   b. Inner shell of egg not bent to form a collar ..... *obsignata* Madsen, 1945.
6. *a.* Ova 65 to 70 μ long; outer shell reticulated. Small intestine of Strigiformes and Falconiformes ..... *falconis-nisi* (Diesing, 1851).
   b. Ova usually less than 62 μ long 7
7. *a.* Ova 46 to 67 μ long (average 53 μ) by 22 to 25 μwide. Worms 11.2 to 20.9 mm long. Ceca of Galliformes ..... *collaris* (v. Linstow, 1873).
   b. Ova 45 μ long by 15 μ wide. Worms 19 mm long. Small intestine of Piciformes .....*longistriata* Walton, 1923.

Males

1. *a.* Lateral caudal alae present 2
   b. Lateral caudal alae absent 4
2. *a.* Bursa heart-shaped, supported by two lobes 3
   b. Bursa supported by two dorsal and two ventral lobes. Spicule sheath transversely striated, without spines. Small intestine of Galliformes .... *bursata*
3. *a.* Spicule sheath transversely striated and provided with minute spines. Small intestine of Galliformes and Passeriformes ..... *caudinflata*
   b. Spicule sheath transversely striated, but without spines. Small intestine of Passeriformes ..... *quiscali*
4. *a.* Spicule sheath spiny 5
   b. Spicule sheath not spiny 6
5. *a.* Tail broad with three lobes. Spicule 1120 to 1840 μ long by 18 to 19 μ wide. Small intestine of Passeriformes ..... *tridens* (Dujardin, 1845).
   b. Tail with two lobes. Spicule 740 to 1890 μ long by 25 μ wide. Ceca of Galliformes ..... *collaris*

6. *a.* Spicule sheath smooth 7
   b. Spicule sheath transversely striated 8
7. *a.* Worms about 11.5 mm long. Spicule 900 μ long, not twisted. Small intestine of Passeriformes ..... *freitasi* n. sp.
   b. Worms about 14 mm long. Spicule 1000 to 1600 μ long, twisted. Intestine of Piciformes ..... *picorum*
8. *a.* Each caudal lobe provided with hook-like papilla on ventral surface. Spicule stout 1500 μ long by 100 μ wide. Small intestine of Piciformes .. *longistriata*
   b. Caudal lobes without hook-like papillae 9
9. *a.* Spicule transversely striated, 750 to 1250 μ long by 12 to 14 μ wide. Small intestine of Strigiformes and Falconiformes ..... *falconis-nisi*
   b. Spicule not striated, expanded at proximal end ..... *obsignata*

Since only the males are known, *C. tridens* and *C. freitasi* are not included in the key to the females.

Not included in the keys are *C. ovopunctatum* and *C. exilis*. Boyd (1946) reported (in abstract) "portions of Capillaria ovopunctatum, C. exilis and immature nematodes" from the examination of 300 starlings from Conn., Ind., Md., Mass., N. Y. and Ohio. The writer is not at all convinced that these two species have been well enough described to allow positive identification from fragments. Therefore, for the present, these are regarded as doubtful records.

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Plate I

Capillaria tridens

Fig. 1. Cauda of male, ventral.
Fig. 2. Spicule, proximal end.

Capillaria falconis-nisi

Fig. 3. Vulva.
Fig. 4. Cauda of male, ventral.
Fig. 5. Proximal portion of spicule, ventral.
Fig. 6. Proximal portion of spicule, lateral.
Fig. 7. Cauda of male, lateral.

Plate II

Capillaria sp. from *Piranga erythromelas*

Fig. 8. Vulva.

Capillaria quiscali

Fig. 9. Vulva.
Fig. 10. Egg.
Fig. 11. Cauda of male, ventral.
Fig. 12. Proximal portion of spicule.
Fig. 13. Distal portion of spicule.

Capillaria freitasi

Fig. 14. Cauda of male, lateral.
Fig. 15. Proximal portion of spicule.

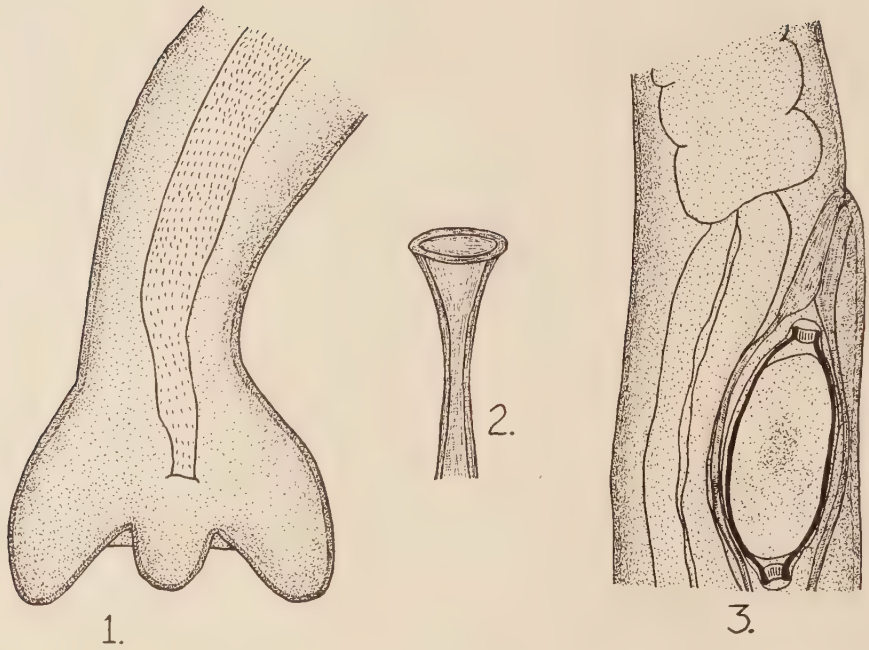

PLATE I

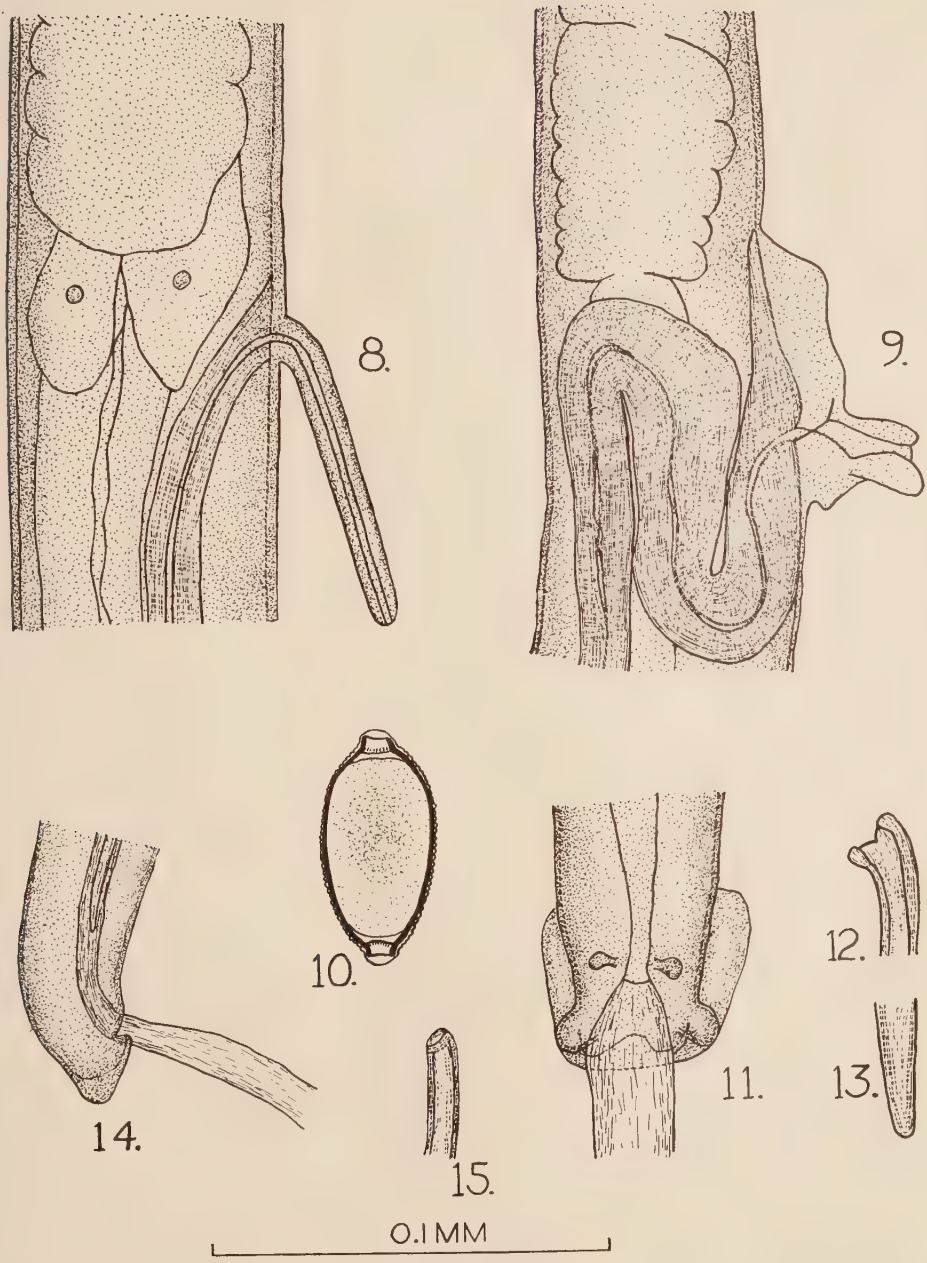

PLATE II

SCHISTOSOME DERMATITIS IN SEATTLE, WASHINGTON

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Otto T. Trott[3] and Elmer V. Howell, Jr.[4]

INTRODUCTION

Interest in schistosome dermatitis in the United States developed largely as a result of the reports by Cort (1928, 1928a) of the disease in Michigan. Subsequently a series of papers were published by Cort and his associates dealing with the various aspects of the disease (La Rue, 1935; Cort 1936, 1936a; Brackett 1940, 1941; Olivier 1947). The presence of what is now recognized as the "*C. elvae* group" was first reported as *C. elvae* by Miller (1925) from San Juan Island in Puget Sound together with *C. tuckerensis*. Miller is reported by Cort (1936a) to have produced two cases of schistosome dermatitis from laboratory exposure to *C. tuckerensis,* which Miller (1925) had described from species of "*Planorbis*" from San Juan Island.

Attention was directed to Green Lake, which lies within the city of Seattle, Washington, because of a newspaper article. This mentioned the existence of a "swimmers' itch" which had been encountered for a number of seasons by persons bathing in Green Lake. The "rash" was of unknown etiology, but it had been suggested that a possible cause was the heavy bloom of algae that occurred during the summer months. With this history, schistosome dermatitis was suspected as the cause and an investigation was initiated.

The present paper is the first record based upon experiments to show the presence of schistosome dermatitis on the mainland in Washington and within the city limits of Seattle.*

EPIDEMIOLOGY

A number of persons who habitually bathed in Green Lake were interviewed. Most of them were children who frequented the bathing beaches or waded along the shore. Many gave a history of being in and out of the water for hours. Picnics were often held at other spots around the lake and in such cases the youngsters were prone to wade there. Consequently it seemed probable that in many cases exposures were not confined to persons swimming at the two bathing beaches which were located on the northeastern and northwestern shores. At both beaches there were concrete retaining walls with steps leading into the water. These areas were covered by fine pebbles or sand and yielded comparatively few snails.

Four species of snails were collected from various shallow water areas around the lake. These snails were segregated by regions and isolated to determine infections. The most abundant species was a lymnaeid, *Stagnicola palustris nuttalliana*

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(Lea)** which was found along the gravel to mud bottom and frequently was associated with reeds and other aquatic vegetation. This species was rarely collected on the beaches, but infected snails were found within 50 to 75 yards of both bathing areas. This snail harbored cercariae of *Trichobilharzia ocellata* (La Valette) (= *Cercaria elvae*), which is known to produce dermatitis. Three of 27 from the east side and 12 of 250 from the west side carried this parasite. It seems likely that these cercariae were carried over the beach area by the wind and currents as has been suggested by Cort (1936) and La Rue (1935) in Michigan.

Physella propinqua (Tryon) was less common, but did occur attached to the cement retaining wall or on pebbles and on sand along the bathing beaches. Only 3 of 42 specimens from the east side and 1 of 62 from the west side were infected by the small, fork-tailed *Cercaria physellae* of *Trichobilharzia physellae* (Talbot).

One hundred and fifty seven *Gyraulus vermicularis* (Gould) and 35 *Menetus planulatus* (Cooper) were collected. These species were not present in quantity at this season of the year and no fork-tailed cercariae were recovered upon isolation. One unidentified species of strigeid cercaria that emerged about 10 P.M. was encountered in *Stagnicola palustris nuttalliana.*

METHODS

Having determined the presence of fork-tailed cercariae in snails collected from Green Lake, it remained to demonstrate which ones, if any, were capable of producing schistosome dermatitis. Two methods were used to test the ability of cercariae to penetrate the skin of human volunteers and to produce schistosome dermatitis. The first consisted of placing a known number of cercariae in small drops of water on the flexor surface of the forearm and permitting evaporation to take place. These spots were ringed with ink and subsequently observed. A second method consisted of placing a known number of cercariae in a petri dish. The flexor surface of the forearm was then placed in contact with the water and the arm was gently rotated for a period of 15 to 30 minutes. The contact with the water was broken at intervals of 2, 5, 10 and 15 minutes and allowed to air dry in order to simulate the periodical immersion of a person playing in and out of the water along a beach. In each case the skin was examined for evidence of macules, petechiae and the individuals checked subsequently to determine whether or not they had experienced any pruritus or other symptoms.

RESULTS

In addition to these experimental laboratory infections, two persons were naturally exposed and developed a marked allergic response when re-exposed approximately one month later. These are described as Cases 1 and 2.

Case 1.—Dr. T. of the Department of Public Health of Seattle helped collect snails 14 June 1947. He collected snails on the north side of Green Lake, wearing bathing trunks, crawling on hands and knees for three hours. The arms to the elbows and the legs were immersed. Itching began an hour after leaving the water and pale macules appeared at the end of six hours. The next morning papules appeared over the exposed areas accompanied by a marked pruritus. Twenty-four

** Grateful acknowledgment is made to Dr. J. P. E. Morrison, Division of Mollusks, U. S. National Museum for the identification of these snails.

hours from the time of exposure there were widely scattered papules 7–8 mm. in diameter with 1–3 elevations over these areas. There was a total of about 20 lesions in all. Benadryl relieved the itching but did not affect the lesions. About a month later while collecting snails in the same area an itching sensation was noticed while still in the water. To quote, "While drying myself vigorously with a towel I noticed that my skin turned red, and in a few minutes my arms and legs burned as though on fire. . . . This was alleviated by the external application of phenol in alcohol and bicarbonate of soda. The next day the legs and arms had the appearance of severe secondary lues. It took 3–4 weeks for the lesions to heal."

Case 2. This case shows the result of a laboratory exposure to *T. ocellata* followed by a natural exposure to unidentified cercariae in Green Lake. On 17 June 1947 Capt. S. exposed a forearm to the cercariae of *T. ocellata* (= *C. elvae*). Slight itching was noted during drying. A few pale macules occurred in 3–4 hours with moderate itching in 7 hours while a few well circumscribed erythematous papules

FIG. 1. Petechiae with surrounding erythematous reaction resulting from an experimental exposure to cercariae of *Trichobilharzia ocellata* 36 hours previously.

were found in the exposed area after 20 hours. No pruritus was noted. Two days later papules 3–8 mm. in diameter with a elevation of 1–2 mm. were seen; these were accompained by pruritus. Subsequently the papules became pale and erythematous with intermittent moderate pruritus for a half hour or longer. Twenty-seven days later snails were collected in the northeast corner of Green Lake (same general area as Case 1). The forearms and right leg were covered with a thin layer of petroleum jelly. The left leg remained unprotected. After 35 minutes exposure the water was allowed to dry without mechanical interference. Faint itching was noted while drying; this became intense in 6–7 hours, with five 8–10 mm. nodules scattered over the non-protected area in 24 hours. These gradually subsided in 5–6 days with intermittent periods of intense itching which lasted for 15–30 minutes at a time.

Laboratory Experiments. Cercariae of *T. ocellata* (= *C. elvae*) from several specimens of *S. palustris nuttalliana* were tested on five volunteers. In all persons characteristic lesions and sequelae were produced, the reactions being more marked in some individuals than in others. One of the more severe reactions (Fig. 1) was

described as follows: "Pruritus following immersion was intense for one hour. In 12 hours there appeared fine erythematous macules with slight papulation and the appearance and feel of rubeola; pruritus becomes intense in 16 hours and is associated with petechiae with surrounding erythematous reactions; the lesions are pale and have the appearance of a foreign body reaction. Pale erythematous papules appear in about a week associated with pruritus and 1–2 mm. elevation with a shotty feeling." (D.D.S.)

Cercariae of *T. physellae* (= *Cercaria physellae*) that emerged from *Physella propinqua* were tested on six volunteers. In four cases pruritus was noted while drying the test area. Subsequently a few lesions typical of schistosome dermatitis developed.

DISCUSSION

Several interesting points may be raised for consideration. It appears that sensitivity can be increased by a second exposure a month later to dermatitis producing cercariae. Whether these must be the same species or whether the reaction occurs after exposure to any of the bird or mammal schistosomes is not yet known. Doctor T. initially reacted strongly on the exposure to what certainly must have included cercariae of *T. ocellata* and a month later reacted violently to re-exposure

TABLE I—*Known Intermediate Snail Hosts of* Trichobilharzia ocellata *and* T. physellae

Parasite	Common synonyms	Snail hosts
Trichobilharzia ocellata, (La Valette)	*Cercaria ocellata*, La Valette 1855 *C. elvae*, Miller 1923 *Trichobilharzia kossarewi*, Skrjabin and Zakharow 1920	*Lymnaea stagnalis jugularis*, Say *L. s. lillianae*, Baker *L. s. sanctaemariae*, Walker *Stagnicola palustris elodes*, (Say) *S. palustris nuttalliana*, (Lea)*
Trichobilharzia physellae, (Talbot)	*Cercaria physellae*, Talbot 1936 *Pseudobilharziella querquedulae*, McLeod 1937	*Physella parkeri*, (Currier) *P. magnalacustris* (Baker) *P. propinqua*, (Tryon)*

* Denotes a new host record.

while collecting in the same area, thus suggesting probable exposure to these same species a second time. Captain S. was known to have been exposed to the cercariae of *T. ocellata* (= *C. elvae*) on the arm in the laboratory. However the second exposure was on the left leg and resulted in a sharp reaction. This suggests that the body may become sensitized in about a month following the initial "stimulating exposure." His exposure also must have included *T. ocellata* cercariae. In addition to ours, other records suggestive of severe allergic responses occur in the literature. For example Cort (1936a) reported the case of a woman in Minnesota who had such a severe reaction that she had to be hospitalized for five days before being able to return home. Olivier (1947) is carrying on an experimental program dealing with this aspect of schistosome dermatitis.

The cercariae were identified on the basis of the size, flame cell pattern, number of penetration glands and the presence of furcal fin folds (Talbot 1936). The final confirmation was made primarily as a result of observations on the time of emergence, tropisms, method of swimming and attachment as described by Cort and Talbot (1936). Similar behavior data are not available on *C. tuckerensis* which was described from *Planorbis* spp. by Miller (1925, 1927). Since this species was originally described from San Juan Island in Puget Sound it might be expected to appear in other areas along the West Coast.

Two new snail hosts are recorded for the schistosome cercariae in Green Lake producing dermatitis, *S. palustris nuttalliana* for *T. ocellata* and *P. propinqua* for *T. physellae* (Table I). However, both records fall into genera already described for these species. It is interesting to speculate whether the "dermatitis-producing schistosome cercariae of the 'elva' group . . . in *L. palustris nuttalliana*" reported by S. G. Saunders in Cort (1936a) is the same species that we encountered in Seattle.

SUMMARY

(1) Cases of schistosome dermatitis are described from Green Lake within the city limits of Seattle, Washington.

(2) Four snails were identified from Green Lake: *Stagnicola palustris nuttalliana* (Lea), *Physella propinqua* (Tryon), *Gyraulus vermicularis* (Gould) and *Menetus planulatus* (Cooper).

(3) Cercariae of *T. ocellata* (La Valette) (= *C. elvae*) and *T. physellae* (Talbot) (= *C. physellae*) were encountered in *S. palustris nuttalliana* and *P. propinqua* respectively, both representing new snail hosts. The first species also harbored an unidentified strigeid cercaria.

(4) What appears to be an allergic response is noted in two cases following sensitization a month previously.

(5) Schistosome dermatitis was produced in human volunteers by both species of schistosomes and not by the strigeid cercariae.

ACKNOWLEDGMENTS

Thanks are gratefully extended to Dr. Cort for suggestions in connection with the manuscript. Thanks are also due to members of the Department of Public Health, City of Seattle, especially to Miss Mulhern, for help in securing data and serving as test hosts, as well as in collecting snails, and to Lt. Col. W. S. Bagnall, MC, C. O. of the Station Hospital, Ft. Lawton for his interest and help on this problem.

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THYROID CONDITION OF CHICKENS AND DEVELOPMENT OF PARASITIC NEMATODES[1,2]

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Considerable recent literature has been devoted to effects of a thyroactive iodocasein (Protamone) and thiouracil on the growth, fattening, and feathering of broilers, *vide* Parker (1943), Irwin, Reineke, and Turner (1943), Turner, Irwin, and Reineke (1944), Kempster and Turner (1945), and Andrews and Schnetzler (1946). One immediate effect of these substances in the diet is exerted upon the activity of the thyroid and when the substances are fed at proper levels it is possible to produce mild hypothyrosis or mild hyperthyrosis in chickens.

The above literature suggested that a further effect of these substances in the diets of chickens might be tested, namely, their effect on resistance to parasitism.

It has been reported, Larsh (1947), that the daily addition of 3 mg of thyroid to the diets of mice resulted in a much higher percentage development of *Hymenolepis* than occurred in control animals. The present writer (1948a, in press) will report significantly superior growth of parasitized chicks with a mild hyperthyrosis when compared with growth of parasitized normal controls, and, when compared with growth of parasitized chicks with a mild hypothyrosis. Wheeler et al (1948) reported that 0.1 percent thiouracil in the diet of 2-week old chicks was not prejudidicial to the survival of birds artificially infected with cecal coccidiosis, while their data on 0.02 percent Protamone in the diet suggested that birds on such a diet were somewhat resistant to the same infection.

The material in this report is concerned with the effect of feeding thyroactive iodocasein and thiouracil on the natural resistance of New Hampshire broilers to infection with two parasitic nematodes, *Ascaridia galli* (Schrank, 1788) and *Heterakis gallinae* (Gmelin, 1790). The report is also concerned with the effect of these substances in the diet of the host on the growth of the two parasites.

MATERIALS AND METHODS

Chicks used in the experiments were straight-run New Hampshires obtained from a commercial hatchery. The day-old chicks were started in electric battery brooders until they feathered, whereupon they were transferred to floor pens in unheated growing quarters where electric hover brooders were used.

The basal ration fed the chicks was similar to the Kentucky starting formula given below:

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[2] The thyroactive iodocasein (Protamone) used in these experiments was supplied by the Cerophyl Laboratories, Kansas City, Missouri; the thiouracil was supplied by the Lederle Laboratories Division of American Cyanamid Co., Pearl River, New York.

	Percent
Ground yellow corn	27
Ground wheat	36
Soybean oil meal	17
Alfalfa meal	2
Dried whey	9
Meat scraps (55 per cent protein)	5
Steamed bone meal	1
Limestone	1.5
Salt	1
Cod liver oil (400 D)	0.5
Manganese sulfate	6 grams

Thyroactive iodocasein (Protamone) was added to the basal ration at a level of 0.04 percent; thiouracil at a level of 0.1 percent. Chicks were started on the hormone diet 3 to 7 days before infection in order that they would become accustomed to the new feed mixtures.

Egg cultures of *Ascaridia galli* and *Heterakis gallinae* were made by removing uteri from gravid females. The uteri were macerated in tap water in clean Petri dishes and, when infective, eggs were administered by means of a pipette inserted into the esophagus of the chicks. Birds infected were given eggs from the same culture in order that all birds might receive eggs of similar age. Known numbers of eggs were given in all cases. The infections were so arranged in each experiment that one-third of the birds received eggs of *A. galli,* one-third received eggs of *H. gallinae,* and one-third received eggs of both worms.

The test periods in the experiments ranged from 21 to 22 days; test periods of such length were adopted to prevent worms from maturing within the infected birds which might have permitted re-infection.

At the conclusion of each test period the birds were killed and examined for parasites; worms found were counted, fixed, and measured.

EXPERIMENTAL RESULTS

Data related to the number of worms which were present in the groups of birds at the termination of the test periods are contained in table I.

In the first experiment birds receiving the special diets were changed from the basal ration at 46 days of age and were infected, along with the control birds on the basal ration only, at 49 days of age. The experiment was terminated when the birds were 71 days old.

In this experiment, in the birds on the basal ration, 8 specimens of *A. galli* and 157 specimens of *H. gallinae* were recovered from the single infections, representing percentage development of 1.1 percent and 15.6 percent, respectively. In the birds on the basal ration which received infections with both worms, 1 *A. galli* (0.1 percent) and 194 *H. gallinae* (23.9 percent) developed.

Birds on the Protamone diet developed 52 *A. galli* (5.9 percent) and 71 *H. gallinae* (15.3 percent) in single infections, and 7 *A. galli* (0.8 percent) and 31 *H. gallinae* (7.7 percent) in the double infections.

Birds on the thiouracil diet developed 6 *A. galli* (1.1 percent) and 173 *H. gallinae*

TABLE 1.—*Percent development of* Ascaridia galli *and* Heterakis gallinae

Experiment and lot	Length test period (days)	Diet	Single Infections								Double Infections						
			Ascaridia galli				*Heterakis gallinae*				*Ascaridia galli* and *Heterakis gallinae*						
			Number birds	Total eggs fed	Total worms recovered	Percent development	Number birds	Total eggs	Total worms recovered	Percent development	Number birds	Total eggs fed	Total worms	Percent development	Total eggs fed	Total worms	Percent development
I B	22	Basal ration	4	745	8	1.1	4	1,007	157	15.6	4	1,001	1	0.1	809	194	23.9
C	22	Protamone	4	874	52	5.9	4	464	71	15.3	4	843	7	0.8	402	31	7.7
D	22	Thiouracil	4	544	6	1.1	4	752	173	23.0	4	1,146	4	0.4	687	255	37.1
II B	21	Basal ration	4	400	9	2.3	4	1,000	26	2.6	4	400	3	0.8	1,000	110	11.0
C	21	Protamone	4	400	2	0.5	4	1,000	218	21.8	4	400	3	0.8	1,000	132	13.2
D	21	Thiouracil	4	400	11	2.8	4	1,000	154	15.4	4	400	5	1.3	1,000	128	12.8

(23.0 percent) in single infections, and 4 *A. galli* (0.4 percent) and 255 *H. gallinae* (37.1 percent) in the double infections.

Numbers of worms collected from birds on the different diets in the first experiment indicated that hyperthyrosis favored the development of *A. galli* and that hypothyrosis favored the development of *H. gallinae*.

In the second experiment birds receiving the special diets were changed from the basal ration at 58 days of age and were infected, along with the control birds on the basal ration only, at 65 days of age. The experiment was terminated when all birds were 86 days old.

In this experiment, in birds on the basal ration, 9 *A. galli* (2.3 percent) and 26 *H. gallinae* (2.6 percent) developed in the single infections, and 3 *A. galli* (0.8 percent) and 110 *H. gallinae* (11.0 percent) developed in the double infections.

Birds on the Protamone diet developed 2 *A. galli* (0.5 percent) and 218 *H. gallinae* (21.8 percent) in the single infections, and 3 *A. galli* (0.8 percent) and 132 *H. gallinae* (13.2 percent) in the double infections.

Birds on the thiouracil diet developed 11 *A. galli* (2.8 percent) and 154 *H. gallinae* (15.4 percent) in the single infections, and 5 *A. galli* (1.3 percent) and 128 *H. gallinae* (12.8 percent) in the double infections.

TABLE 2.—*Size of* Ascaridia galli *and* Heterakis gallinae *in chickens fed basal ration, protamone, or thiouracil*

Experiment and lot	Nature of diet	Single Infections				Double Infections				Age of all worms (days)
		A. galli		*H. gallinae*		*A. galli*		*H. gallinae*		
		Number worms	Average length (mm)	Number worms	Average length (mm)	Number worms	Average length (mm)	Number worms	Average length (mm)	
I B	Basal ration	8	3.3	157	4.2	1	11.3	194	4.9	22
C	Protamone	52	30.5	71	2.4	7	6.9	31	3.3	22
D	Thiouracil	6	7.2	173	5.5	4	6.2	255	6.2	22
II B	Basal ration	9	6.0	26	2.0	3	7.2	110	3.9	21
C	Protamone	2	15.0	218	2.5	3	17.1	132	2.4	21
D	Thiouracil	11	6.3	154	4.2	5	4.1	128	4.4	21

Numbers of worms collected from birds on the different diets in the second experiment indicated, in contrast to the results of the first experiment, that hyperthyrosis favored development of *H. gallinae* and that hypothyrosis favored development of *A. galli*.

In summary, in the two experiments 2.5 percent of *A. galli* eggs developed in birds fed Protamone, 1.0 percent developed in birds receiving thiouracil, and 0.8 percent developed in birds on the basal ration. In the same experiments, 20.6 percent of infective eggs of *H. gallinae* fed to birds on thiouracil developed, 15.7 percent developed in birds fed Protamone, and 12.7 percent developed in birds fed the basal ration.

When the percentages of development of the 2 species of worms were isolated and compared by means of an analysis of variance they were found not to be significant.

Data related to the length of the worms recovered from parasitized birds are contained in table 2.

Specimens of *A. galli* recovered from birds receiving Protamone were longer, consistently, than those recovered from birds receiving thiouracil, in some instances averaging 4 times as long. Specimens of *H. gallinae* recovered from birds receiving

Protamone were shorter, consistently, than those recovered from birds receiving thiouracil. In brief, in the 2 experiments where both Protamone and thiouracil were used, 64 specimens of *A. galli* from birds on Protamone averaged 26.8 mm in length; 26 *A. galli* from birds on thiouracil averaged 6.1 mm long. A total of 452 *H. gallinae* from birds on Protamone averaged 2.5 mm long, while 710 specimens from birds on thiouracil averaged 5.3 mm.

Specimens of *A. galli* recovered from birds on the Protamone diet were larger than those recovered from birds on the basal ration. A total of 21 *A. galli* from birds on the basal ration averaged 5.4 mm long.

Specimens of *H. gallinae* recovered from birds on the Protamone diet were smaller than those recovered from birds on the basal ration. The 452 *H. gallinae* recovered from birds on Protamone averaged 2.5 mm long. A total of 487 *H. gallinae* from birds on the basal ration averaged 4.3 mm long.

A comparison of worms recovered from birds on thiouracil with worms recovered from birds on the basal ration, shows that the 26 *A. galli* from birds on thiouracil averaged 6.1 mm long and the 21 *A. galli* from birds on the basal ration averaged 5.4 mm long. The 710 *H. gallinae* from birds on thiouracil averaged 5.3 mm long and the 487 specimens from birds on the basal ration averaged 4.3 mm long.

In summary, specimens of *A. galli* were larger in birds on Protamone than in birds on thiouracil or the basal ration. Specimens of *H. gallinae* were largest in birds on thiouracil and smallest in birds on Protamone. When the above differences in mean length of the two species of worms were submitted to an analysis of variance they were found to be significant. The greater size of *A. galli* in mildly hyperthyroid hosts was found to be significant and the greater size of *H. gallinae* in mildly hypothyroid hosts was found to be highly significant.

DISCUSSION

Because hypothyrosis and hyperthyrosis are relative and because such conditions can readily be produced by changes in the diet of chickens, the type of investigation here reported has an immediate application in the field of applied parasitology. Ackert et al (1935) found heavier breeds of chickens, such as Rhode Island Reds, White and Barred Plymouth Rocks, more resistant to infection with *A. galli* than White Leghorns and White Minorcas. The present writer (1948b) reported a survey of worm populations of chickens in Tennessee where greater numbers of *H. gallinae* occurred in heavy breeds. One significant difference between heavy and light breeds of chickens is generally considered to be the level of thyroid activity. Worm populations in Tennessee chickens were found to be conditioned by a typical parasite fauna available at each poultry farm. It appears, in view of Larsh's report (1947), that level of thyroid activity might inhibit, or promote, development of individual species of parasites and condition measures designed to control infections. Development of *A. galli* and *H. gallinae* was not found here to be significantly different in mildly hypothyroid or hyperthyroid chickens.

The significantly greater lengths attained by *A. galli* in mildly hyperthyroid chickens, and the significantly greater lengths of *H. gallinae* in mildly hypothyroid chickens, appear to be the first reports of host thyroid conditions influencing size of parasitic worms.

SUMMARY

A study has been made of the effect of thyroactive iodocasein and thiouracil in the diet of New Hampshire broilers on the natural resistance of the host to infection by *Ascaridia galli* and *Heterakis gallinae,* and, of the effect of the two substances on the growth of the two parasitic worms.

No significant differences in percent development of either worm were found in mildly hyperthyroid, mildly hypothyroid, or normal hosts.

Specimens of *A. galli* attained significantly greater lengths in mildly hyperthyroid birds. Specimens of *H. gallinae* attained significantly greater lengths in mildly hypothyroid birds. These appear to be the first reports of host thyroid conditions influencing size of parasitic worms.

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THE COMPARATIVE EFFECT OF SOME SULFONAMIDES ON EXPERIMENTAL TRICHINOSIS IN WHITE MICE[1]

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In recent years it was demonstrated that some sulfonamides possess remarkable prophylactic and therapeutic value against certain bacterial and protozoan diseases. In a very able review Van Someren (1939) listed substances tried for the treatment of trichiniasis. None of the substances reported were very satisfactory.

Sulfanilamide has been studied in the treatment of trichinosis but the results of the investigators are contradictory. McCoy (1938) presented results taken from two experiments each consisting of experimental and control groups of rats. The experimental rats were treated for 12–16 days with daily doses of 125 mg of sulfanilamide. Chemotherapy began one day after trichinization. When the rats were autopsied 30–60 days after parasitism he found that the sulfanilamide treated rats harbored about as many muscle cysts as the control rats. For a limited study of sulfanilamide therapy in trichinosis McNaught *et al* (1939a) incorporated sulfanilamide in a ration at a concentration of 1.66 per cent and fed it to rats for 42 days beginning two days prior to trichinization. They found that the four rats fed the sulfanilamide ration harbored only 45 per cent as many muscle larvae as the four control rats fed the same ration without the sulfanilamide.

The investigation herein reported was performed to test further the effectiveness of sulfanilamide upon *Trichinella spiralis* and make a comparative study of the effects of other commonly used sulfonamides on the parasite.

METHODS

The hosts used in testing the comparative effectivity of sulfonamides in the control of *Trichinella spiralis* were mature white mice. In order to eliminate the differences in parasite numbers which might result from age and sex variations of the host, the mice in each experiment were separated into groups so that for each mouse in the control group there was a mouse of the same age and sex in each of the other five groups.

Following a short period of starvation each mouse was individually parasitized by placing a small morsel of rat diaphragm containing 100 ± 5 microscopically counted *T. spiralis* cysts into its mouth with a slender, blunt forceps. Beginning shortly after parasitism until autopsied the groups of mice were fed their respective diets consisting of water and one of six rations prepared as described below.

The chief factor in sulfonamide therapy is to maintain a constant level of blood and tissue concentration of the drug where the parasite lives. Since this factor depends somewhat upon the method of administering the drugs, the pharmacology of the sulfonamides tested had to be carefully reviewed. It was decided to administer

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the sulfonamides by incorporating them in the ration (Bieter *et al,* 1939, 1940) rather than in the water (Seastone, 1937) or at frequent daily intervals (Feinstone *et al,* 1938). It was thought too inconvenient and disturbing to the host to administer the drug individually several times daily throughout the long therapy period, and in the water it was doubted that the host would get sufficient quantities of some of the sulfa drugs since Fox and Rose (1942) reported a low solubility rate for some of the sulfonamides to be tested. Incorporating the drugs in the ration appeared likely to be the most feasible method of administration, because the frequent and regular feeding habit of the mouse insured the presence of sufficient quantities of the various sulfonamides in the intestine to maintain a desirable constant blood and tissue concentration.

It is known that an over-dose of sulfonamide may cause hepatic disturbance and crystallization of the drug in the renal tubules followed by renal obstruction and dilation. It was necessary, therefore, to determine the maximum amount of each sulfonamide that could be added to the ration without reducing the palatability of the latter and provide a dose sufficiently small to be nonlethal, nontoxic and nonpathological during the entire therapy period. The work by Richardson (1941) and Hac *et al* (1942) on dosage and relative blood concentrations of sulfonamides and the investigations on toxicity and pathology by Hac and Eilert (1943) on sulfanilamide, Powell and Chen (1941) and Marshall and Litchfield (1939) on sulfapyridine, Marshall *et al* (1940) on sulfaguanidine, McKee *et al* (1939) and Marshall *et al* (1940) on sulfathiazole and Feinstone *et al* (1940) on sulfadiazine were instrumental in determining the quantities of the sulfonamides to incorporate in the rations. The rations were prepared and fed to designated groups of mice at the following concentrations:

Group I—2% sulfanilamide
Group II—1% sulfapyridine
Group III—2% sulfaguanidine
Group IV—1% sulfathiazole
Group V—0.5% sulfadiazine
Group VI—Control —No drug

The adults and muscular larvae served as criteria for determining the effectiveness of sulfonamides in controlling trichinae. Six days after parasitism the adult trichinae were obtained by removing the small intestine and caecum of each mouse These organs were slit, cut into short pieces and refrigerated in a solution of one per cent sodium hydroxide. After the mucus had dissolved, the adults were counted directly with a dissecting microscope.

Four weeks after parasitism the remaining animals were autopsied, skinned and eviscerated. The remainder of each carcass was chopped into small pieces and digested at 37° C in 25 grams of artificial gastric juice per gram of flesh.

After digestion was completed the gastric juice was highly diluted and set aside until the larvae had settled. The supernatant water was then drawn off. This process was repeated until the liquid containing the larvae was clear. The larvae were then concentrated and counted with a dissecting microscope in five separate 0.5 cc samples of the solution on a hookworm, egg-counting slide. To determine the total number of larvae harbored by each mouse, the mean of these counts was

TABLE 1.—*The adult* T. spiralis *harbored by the mice in the first experiment on the sixth day after parasitism with 100 ± 5 cysts*

Sulfanilamide	Sulfapyradine	Sulfaguanidine	Sulfathiazole	Sulfadiazine	Control
13	37	42	24	46	26
47	36	16	21	6	15
68	73	25	25	26	22
36					
Average 41	49	28	23	26	21

multiplied by twice the total number of cubic centimeters of solution containing the larvae.

EXPERIMENTAL RESULTS

The results on the effectiveness of sulfonamides in the control of *Trichinella spiralis* in mice infected with 100 ± 5 cysts are represented by the first four tables. Tables 1 and 2 represent the results of experiment one; Tables 3 and 4 represent the results of experiment two.

The average numbers of adult trichinae harbored by the sulfonamide fed groups of mice in Table 1 were no smaller than the average number of worms harbored by the control group of mice. This indicated that the presence of sulfanilamide,

TABLE 2.—*The numbers of larvae harbored by the mice in each of the diet groups of the first experiment following 28 days of parasitism with 100 ± 5* T. spiralis *cysts*

Sulfanilamide	Sulfapyradine	Sulfaguanidine	Sulfathiazole	Sulfadiazine	Control
14000	30280	14520	20880	32480	26000
13880	32640	15400	16520	19200	14540
15480	31840	22880	25600	20600	32000
13600	6000	52000	33240	20800	9040
7040	23600	22640	27080	40800	24800
9000	26400	27480	29280	21640	48800
			24640	26800	28880
Average 12160	25127	25820	25320	26046	26294

sulfapyridine, sulfaguanidine, sulfathiazole, or sulfadiazine in the diets were not effective in reducing the numbers of adult trichinae harbored by the mice during the first six days of parasitism.

The control group of mice, Table 2, yielded an average of 26,294 larvae. The average numbers of larvae yielded by the mice fed the diets containing sulfapyridine, sulfaguanidine, sulfathiazole or sulfadiazine were about the same as that of the control group. The sulfanilamide group of mice had an average of only 12,160 larvae. This was a larval reduction of 53.8 per cent. Although variations appeared within the groups, the average numbers of larvae harbored by all the groups of mice except the sulfanilamide group were fairly uniform. This indicated that the reduced

TABLE 3.—*The numbers of adult trichinae harbored by the mice of experiment 2 after a 6-day period of parasitism with 100 ± 5 cysts*

Sulfanilamide	Sulfapyradine	Sulfaguanidine	Sulfathiazole	Sulfadiazine	Control
9	52	44	65	45	56
12	35	44	35	54	58
13	49	26	43	50	49
12	34	27	51	13	25
		6	23	75	
Average 12	43	29	43	47	47

average number of larvae harbored by this group was caused by the presence of the drug in the diet and not by chance.

The recovery of adult trichinae in the second experiment is shown in Table 3. The average numbers of adult worms harbored by the control and sulfadiazine groups of mice were larger than those of the other groups. The sulfanilamide group had an average of only 12 worms per mouse; the sulfaguanidine group average was 29 worms per mouse. The average worm numbers harbored by the other sulfonamide groups of mice were only slightly lower than that of the control group. This indicated a marked reduction of adult trichinae harbored by the mice in the sulfanilamide group and some reduction in the sulfaguanidine group.

From Table 4 it may be noted that the average number of larvae of the sulfanilamide group of mice was 9,196 compared to the average number of 20,232 larvae for the control group. The sulfaguanidine group of mice yielded an average of 13,456 larvae per mouse. This was an average larvae reduction of 55 per cent for

TABLE 4.—*Showing the numbers of larvae harbored by the mice in each of the sulfonamide and control groups autopsied 28 days after parasitism with 100 ± 5* T. spiralis *cysts*

	Sulfanilamide	Sulfapyradine	Sulfaguanidine	Sulfathiazole	Sulfadiazine	Control
	7200	17840	11600	16200	22160	40400
	5280	26640	12280	22400	12000	14480
	8500	14720	3200	21080	27040	11600
	14200	16800	15400	11880	15540	15480
	10800	28640	24800	27400		19200
Average	9196	20928	13456	19792	19185	20232

the sulfanilamide group of mice and a 33.5 per cent larvae reduction for the sulfaguanidine mice.

DISCUSSION

Examination of the results on the comparative effect of sulfonamides in the control of trichinae revealed that only sulfanilamide was markedly effective. The larval reduction in the sulfanilamide groups of mice of the two experiments in this investigation was found to be 54 per cent less than the over-all, larva number yielded by the control mice. This corresponded closely to the 55 per cent larva reduction obtained by McNaught *et al* (1939a) when they investigated the effect of sulfanilamide on trichinized rats.

Although the mice fed the sulfaguanidine ration in the first experiment harbored as many trichina adults and larvae as the controls, the same group of the second experiment showed a reduction of 34 per cent larvae and 38 per cent adults. Whether this reduction in adults and larvae in the second experiment was caused by the presence of sulfaguanidine in the diet may be determined through additional experimentation.

Even though the mice were parasitized with 100 ± 5 *T. spiralis* larvae, the results indicated a rather wide range in the numbers of adult and larva parasites harbored by the hosts within the groups. These differences may be attributed to several factors. The mice were evenly separated as to age and sex among the experimental groups, age and sex differences did exist within each group. The wide range in the numbers of parasites habored by the mice within each group might have been caused by either age or sex differences since Riedel (1948) showed that

older mice were less susceptible to trichina larval infections than young mice, and McNaught *et al* (1939b) reported females to be more resistant than males. Variations in numbers of trichina parasites harbored by hosts of the same age and sex were thought by Miller *et al* (1932) to be caused by biological variations of the hosts.

No signs of toxicity resulting from the drugs or symptoms of trichinosis were observed in any of the groups of mice during the entire experimental period.

SUMMARY

The effect of several sulfonamides on trichinae was studied. The sulfonamides were incorporated in rations at nontoxic levels. These rations were fed to two series of mice immediately after infection with 100 ± 5 *T. spiralis* cysts until autopsied. When the numbers of parasites harbored by the sulfonamide fed groups of mice were compared to the numbers of parasites harbored by the control mice, it was found that sulfanilamide fed to mice at a two per cent concentration caused an overall larval reduction of 54 per cent. The effect of sulfaguanidine on trichinae at a two per cent level was slight and doubtful. Rations containing one per cent sulfapyridine, one per cent sulfathiazole or 0.5 per cent sulfadiazine were ineffective on the parasite.

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THE EARLY LARVAL STAGES OF *SCHISTOSOMA MANSONI* SAMBON, 1907 IN THE SNAIL HOST, *AUSTRALORBIS GLABRATUS* (SAY, 1818)

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In a series of related studies Cort and his associates have presented information on the early larval stages of trematodes belonging to the groups Strigeata (Cort and Olivier, 1941), Schistosomatoidea (Cort and Olivier, 1943b; Cort, Ameel, and Olivier, 1944), Plagiorchioidea (Cort and Olivier, 1943a; Cort and Ameel, 1944), and the Fasciolatoidea (Cort, Ameel, and Van der Woude, 1948). In these investigations the morphology of the larval stages was studied in considerable detail and special emphasis was placed on determination of the method of reproduction. Prior to these studies, detailed information on the early developmental stages of trematodes in the groups mentioned was very meager.

Two of the studies dealt with schistosomes. Cort and Olivier (1943b) reported on the early developmental stages of *Trichobilharzia stagnicolae* (Talbot, 1936). It was shown that the mother sporocysts are relatively large, elongate, sausage-shaped sacs usually located in the head-foot region of the snail. They produce numerous daughter sporocysts by means of multiplication of the germinal cells (primary polyembryony) and by a special form of polyembryony involving germinal masses[2] (secondary polyembryony). In the daughter sporocysts the cercarial embryos are also produced by multiplication of the germinal cells and by polyembryony involving germinal masses in the same manner as the daughters themselves were produced.

In the second of the two studies dealing with schistosomes Cort, Ameel, and Olivier (1944), desribed the developmental stages of *Schistosomatium douthitti* (Cort, 1914). They found that division of the germinal cells in the miracidium results in production of about 200 germinal cells in the young mother sporocyst. Each of these germinal cells develops into one daughter sporocyst. These daughters leave the mother and migrate to the digestive gland. Germinal cells carried in the daughter sporocysts multiply until about 200 cells result. Most of these cells develop into cercariae, but a few give rise to germinal masses which in turn produce cercariae. No germinal masses were seen in the mother sporocysts and only a few were seen in the daughter sporocysts, suggesting that polyembryony by germinal masses in this species is relatively uncommon.

It was felt desirable to compare the development of the schistosomes of man with that described for *T. stagnicolae* and *S. douthitti.* There is little available information on the early larval stages of *Schistosoma haematobium* and *Schistosoma japonicum.* Only Maldonado and Acosta-Matienzo (1947) have presented detailed in-

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[2] Cort, Ameel, and Van der Woude, adopted the term "germinal mass" to replace "germ mass" which was used previously for these structures. Their revised terminology has been adopted here.

formation on the early developmental stages of *Schistosoma mansoni,* and they did not attempt to relate their findings to the available data on *T. stagnicolae* and *S. douthitti.* Moreover, their data and figures do not lend themselves well to such a comparison. Consequently, a study of the larval stages of *S. mansoni* was undertaken to supplement the observations of Maldonado and Acosta-Matienzo and especially to gather data that would be useful in comparing the development of *S. mansoni* with that of the other two species. Pertinent results of this study are presented here. Since many of the observations made do not represent new information, they are not included except when it is felt desirable to confirm previous findings.

METHODS

All of the observations were made on a strain of *S. mansoni* originally obtained from Puerto Rico and maintained in the laboratory in mice and hamsters. The snails in which the infections were studied, laboratory-reared *Australorbis glabratus* (Say, 1818), also came from Puerto Rican stock. All snails were infected when young and not over 5 mm in diameter. No difficulty was experienced in infecting the snails and in some of the experiments all of the snails became infected. Multiple infections were common whenever numerous miracidia were used to infect the snails. Some of the data came from single-miracidium infections. All recorded observations were made from living specimens teased from the snail or studied in place within the snail tissue. Fixed material was considered unsatisfactory because of distortion resulting from fixation and because of the disadvantages inherent in reconstruction from serial sections. Also, there was considerable advantage in being able to manipulate living specimens under the cover glass. For instance, relationships of sporocyst contents were often clarified by subjecting living specimens to cover-glass pressure. The finer details of structure were studied under magnifications up to 970 diameters. Nile blue sulfate and neutral red were of value in emphasizing certain larval characteristics but most of the observations were made on unstained specimens. The mother sporocysts could not be dissected free from the snail tissue and so had to be studied in place, but the contents of the mothers were dissected free for study. The daughter sporocysts could be dissected free from the tissue when very young but after their establishment in the digestive gland it was much more difficult to remove them from the snail tissue. Many specimens in all stages of development, from young mother sporocysts to mature cercaria-producing sporocysts, were studied.

Developmental stages of *Schistosoma mansoni* in the snail host

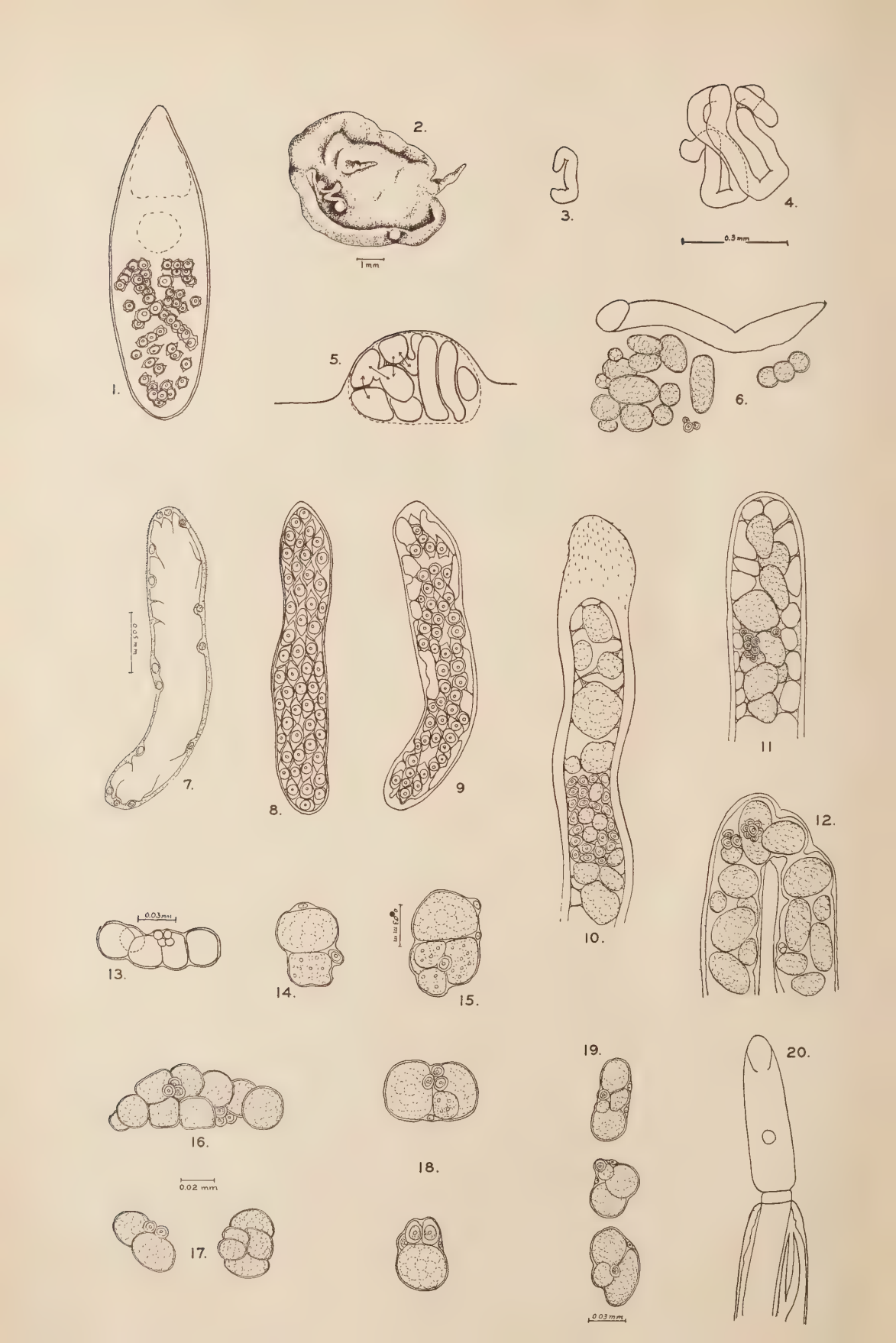
Germinal cells in the miracidium: The germinal cells in miracidia of *S. mansoni* were counted in order to determine the approximate amount of germinal material available when the snail phase of the infection begins. Counts from 8 miracidia ranged from 38 to 61 (average 53). The germinal cells were distributed throughout the posterior two-thirds of the miracidium (Fig. 1). It was noted that in some of the miracidia several of the germinal cells were conspicuously larger than the others, being about 5 to 6 microns in diameter, whereas most of the cells were about 4 microns in diameter. Except for their size, these larger germinal cells were indistinguishable from the others.

Mother sporocysts: By the end of the second week of development the mother sporocysts near the body surface became readily visible under low magnification as small protuberances from the surface that were usually paler than the associated snail tissue. The size of these tumefactions was not uniform but they were approximately 0.5 mm across. When the sporocysts developed in the tentacles they caused relatively great distortion and were very conspicuous. The locations of 95 mother sporocysts seen in the study were recorded. Of these, 30 were located near the surface of the head-foot, 26 were on the mantle, 25 were on the tentacles, 10 were in the pseudobranch, 3 were on the respiratory membranes, 1 was near the brain, and 1 was on the surface of the stomach.

The basic shape and structure of the mother sporocysts was discernible during the first two weeks of development. Figures 3, 4, and 5 show three stages in the early development of mother sporocysts. The youngest of the three (Fig. 3), 7 days old, was dissected from the edge of the mantle in a mass of snail tissue. It was a simple sac about 0.25 mm long filled with germinal cells. Another mother sporocyst (Fig. 4), 9 days old, occupied a spherical area about 0.3 mm in diameter in the tissue near the base of a tentacle. It was much longer than the 7-day old sporocyst and much convoluted, yet the loops could be traced completely and it was evident that it was a simple, elongate sac. The third mother sporocyst (Fig. 5), 14 days old, was found on the edge of the mantle. It occupied a larger space and filled that space almost completely with its tightly packed loops. Nevertheless, connections between some of the distended portions of the mother sporocyst were seen clearly. Older mother sporocysts were so packed with larvae, so tightly convoluted, and so firmly embedded in the host tissue that their organization and contents could not be studied while they were intact. However, there was no evidence that they divide transversely.

Mother sporocysts were seen in many infections in which there were young developing daughters in the digestive gland but they were usually not found in older, more mature infections. For instance, in one infection from a single miracidium the digestive gland contained numerous daughters with cercarial embryos in the spherical "germ ball" stage and the mother sporocyst, containing 78 small embryos and elongate daughters, was found on the side of the foot. On the other hand, in two single-miracidium infections producing fully developed cercariae the mothers were degenerate and contained only a few daughters about 0.1 mm long, several smaller embryos, and possibly a few separate germinal cells.

Contents of mother sporocysts: Young mother sporocysts such as those in Figure 3 contained only single germinal cells, while older mother sporocysts also contained embryo daughters in increasing proportion. The 9-day old mother sporocyst, mentioned previously, contained numerous single germinal cells among which were scattered small embryos each composed of from 2 to 8 cells. The sporocyst contents were so crowded that the relationship between the germinal elements and the wall of the sporocyst could not be determined. The 14-day old mother sporocyst (Fig. 5) contained many embryos of various sizes, some of which were elongate daughter sporocysts up to 0.2 mm in length. All of the germinal elements, except single germinal cells, were counted in three mother sporocysts that contained elongate daughters which appeared ready to emerge. These mothers apparently had shed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no daughters since none of the latter could be found in the snail tissue. Counts of 208, 258, and 299 were obtained in the three cases; these counts are probably slightly smaller than the actual number of multicellular elements in the sporocysts since a few were probably destroyed or lost in dissecting the mother sporocyst for removal of the embryos.

Although careful search was made, no germinal masses were found in mother sporocysts of *S. mansoni*. Escape of the daughters from the mothers was not observed.

Daughter sporocysts about to leave the mother: The largest daughter sporocysts found inside mother sporocysts were essentially elongate, thin-walled sacs containing numerous single germinal cells. The surface of these daughters was covered with spines which were quite obvious anteriorly but smaller and very inconspicuous posteriorly. The wall of the daughter was made up of a thin cuticula overlying a thin layer of cells which are readily differentiated from the germinal cells within the lumen because of differences in size and structure of the nucleus (Fig. 7). Of the daughters found inside the mother, the largest individuals were 0.15 to 0.24 mm long and their width was roughly one-sixth of their length. Ten of these daughters contained 50, 53, 58, 60, 63, 66, 75, 88, 112, and 130 germinal cells

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EXPLANATION OF PLATE

All drawings were made from living specimens.

FIG. 1. Sketch of a miracidium of *Schistosoma mansoni* showing number and location of the germinal cells, three of which cells are distinctly larger than the others. (Drawn from a flattened specimen).

FIG. 2. Head-foot of a snail showing four mother sporocysts visible from the outside as small protuberances on the tentacle, mantle, and pseudobranch.

FIG. 3. Mother sporocyst of *S. mansoni* 7 days old and about 0.25 mm long. Drawn to same scale as Figures 4 and 5.

FIG. 4. Mother sporocyst of *S. mansoni* 9 days old. Shows elongate, tubular nature of the sporocyst and tendency to become tightly convoluted.

FIG. 5. Optical section through a mother sporocyst of *S. mansion* 14 days old showing portions of the long, convoluted sporocyst. Indications of the tubular nature of the sporocyst are evident.

FIG. 6. Typical contents of a mother sporocyst of *S. mansoni*. One elongate daughter, small embryos, and several separate germinal cells.

FIG. 7. Wall of a daughter sporocyst of *S. mansoni* which is in the migrating stage. Optical section. Shows nature of the cells of the wall.

FIG. 8. Daughter sporocyst of *S. mansoni* in the migrating stage showing the number and arrangement of the germinal cells. Flattened specimen. Optical section.

FIG. 9. Same sporocyst as in Fig. 7. Shows complete germinal content. Spaces between the cells are due to flattening under heavy cover glass pressure. The germinal cells are apparently connected by protoplasmic processes.

FIG. 10. Portion of a young daughter sporocyst of *S. mansoni* from the digestive gland. The sporocyst contains small separate cercarial embryos and a mass containing germinal cells and very small embryos.

FIG. 11. Portion of a young daughter sporocyst of *S. mansoni*. Contents are further developed than those of Fig. 10. The cercarial embryos are larger but there are single germinal cells present.

FIG. 12. Portion of a daughter sporocyst of *S. mansoni*. Contents are further developed than those of Fig. 11. Cercarial embryos are oval and only a few germinal cells are present.

FIGS. 13, 14, 15, 16, 17, 18, and 19. Germinal material from daughter sporocysts of *S. mansoni*. Figures 13, 14, 15, 18, and 19 are germinal masses with both unicellular and multicellular components. Each is enclosed by a distinct membrane. Figures 16 and 17 show similar sporocyst inclusions but they are not enclosed by a membrane.

FIG. 20. End of a daughter sporocyst of *S. mansoni* with a cercaria emerging through the terminal birth pore.

respectively. These cells were all approximately the same size and in undistorted specimens almost completely filled the lumen of the daughter sporocysts (Fig. 8). When the daughters were subjected to cover glass pressure, spaces between the cells were sometimes seen (Fig. 9). The germinal cells did not change their relative positions when the sporocysts moved. Also, in specimens flattened under cover glass pressure the cells seemed to be joined by strands of protoplasm.

Daughter sporocysts after leaving the mother: Daughter sporocysts, indistinguishable from the largest daughters found inside mother sporocysts, were recovered from the digestive gland and other organs of snails 19 days or more after the snail was exposed to infection. These contained only single germinal cells. Larger daughter sporocysts in the digestive gland contained, in addition to single germinal cells, small cercarial embryos of various sizes. This germinal material was usually crowded but in some daughters there were small spaces between the germinal elements. In these cases the germinal cells and small embryos seemed to be adherent to one another and to the wall of the sporocyst by means of protoplasmic strands. Several daughters in this stage of development contained a large mass of single germinal cells or a mass of both single germinal cells and small cercarial embryos. This mass filled the lumen of the sporocyst (Fig. 10). In older sporocysts containing larger embryos that were beginning to elongate, the germinal elements were usually free in the lumen.

Most of the daughter sporocysts were found in the digestive gland but it is noteworthy that in some infections, and particularly in heavy ones, daughter sporocysts were found in other locations such as in the respiratory membranes and on the surface of the stomach and other organs. In one instance, a daughter containing fully developed cercariae was found at the ventral surface of the foot where it was mistaken, at first, for a mother sporocyst since it appeared as a small, pale spot readily visible under low magnification.

Germinal masses in the daughter sporocysts: Most of the daughter sporocysts which were studied contained single germinal cells and cercarial embryos in varying proportions depending on their stage of development. However, typical germinal masses were also present in a small number of the daughter sporocysts. These masses were composed of single germinal cells and multicellular components and each was enveloped in a distinct membrane composed of one or more flattened cells (Figs. 13, 14, 15, 18, 19). These germinal masses were not seen consistently and none could be found in many of the infected snails; on the other hand, in a few of the infected snails they were found in considerable numbers.

Escape of the cercariae from the daughter sporocysts: The development of the cercariae was not studied, but escape of the cercariae from the daughter sporocysts was observed on several occasions. When the shell and tunica propria of the snail were dissected away from the digestive gland of an infected snail, portions of the daughter sporocysts could usually be seen on the surface of that organ as whitish, worm-like bodies. Closer inspection showed that some of the visible portions of the parasites were sporocyst ends protruding from the surface of the gland. These ends changed shape frequently due to activity of the cercariae inside, and on several occasions a cercaria was observed to leave by slowly working its way out through a birth pore in the tip of one of these protruding portions. The birth pore is termi-

nal and inconspicuous and was not seen except when a cercaria was in the act of leaving the sporocyst (Fig. 20).

DISCUSSION

The germinal material brought to the snail by the miracidium of *Schistosoma mansoni* consists of a small number of cells. These unorganized cells of the germinal and inconspicuous and was not seen except when a cercaria was in the act of cells counted in the miracidia in the present study agrees closely with the number reported by Maldonado and Acosta-Matienzo (1947).

The observations on the mother sporocysts made in the present study confirm in large measure those reported by Maldonado and Acosta-Matienzo for this stage. No evidence was found to indicate that the mother sporocysts migrate after they begin to develop in the snail, and it is also clear that the mother sporocysts usually develop close to the point of penetration of the miracidium since most of the mother sporocysts were on or near the surface of the snail. The length of life of the mother sporocysts was not determined. Available evidence indicates that they live for some time after the first daughters are produced but not longer than the time at which cercariae begin to emerge from the snail. Escape of daughters from the mother was not observed, but there is no evidence in the present study indicating that they escape by rupturing the wall of the mother as described by Maldonado and Acosta-Matienzo. It is the writers' opinion that there is probably a terminal birth pore similar to that known to occur in mother sporocysts of *Trichobilharzia stagnicolae* and *Schistosomatium douthitti.*

The number of daughter sporocysts produced by a mother sporocyst of *S. mansoni* is variable but the usual number is probably between 200 and 400. Maldonado and Acosta-Matienzo state that "hundreds" are produced. The largest number of germinal elements counted by us from one mother sporocyst was 299, but in this count some of the embryos may have been missed and single germinal cells, of which a small number may have been present, were not counted. Apparently the daughter sporocysts are produced simply by multiplication of the germinal cells carried in the miracidium. Division of these cells within the lumen of the developing mother sporocyst results first in numerous germinal cells that fill all of the lumen. Later these cells develop into daughter sporocyst embryos by repeated cleavages. No germinal masses were seen in the mother sporocysts of *S. mansoni.* The possibility remains that germinal masses may occur in that stage but, if they do, their number must be very small and they could have little significance in the production of daughter sporocysts.

When the daughter sporocysts of *Schistosoma mansoni* are ready to leave the mother sporocyst they usually contain 50 to 100 germinal cells or roughly the same number of germinal cells as the miracidium carries to the snail. In other words, at this stage there has been a 200- to 400-fold reproduction.

As Maldonado and Acosto-Matienzo have shown, there is usually no development of the daughters while they are migrating. The smallest daughters in the digestive gland are the same size as the largest still in the mother. Daughters which have developed farther than this stage are sometimes found outside the digestive gland, but this does not necessarily mean that they undergo development during migration since some of the daughters complete their development in organs other than the digestive gland.

No specific information was obtained concerning the number of cercariae that may be produced by one daughter sporocyst of *S. mansoni.* Faust and Hoffman (1934) report that more than 210,000 cercariae may be produced from one miracidium. Since only 200 to 400 daughter sporocysts are produced in the mother sporocysts of this species, and these usually contain less than 100 germinal cells each, it is apparent that there is extensive multiplication within the daughters after they leave the mother.

Most of the cercariae of *S. mansoni* arise through division of the germinal cells without intervention of germinal masses since few germinal masses were seen in daughter sporocysts. This multiplication comes about primarily through the division of the germinal cells resulting in numerous separate germinal cells each of which may develop into a cercaria. However, since some germinal masses were observed, it appears that some cercariae may be produced by this means. Perhaps the germinal masses occur at only a short period in the life cycle, or perhaps they arise only as a response to unusual conditions. At any rate, it appears certain that the germinal masses are of less significance in the life cycle of *S. mansoni* than they are in the life cycle of *T. stagnicolae.*

The development of *Schistosoma mansoni* in the snail parallels closely that described for *Trichobilharzia stagnicolae* and *Schistosomatium douthitti.* In all three the miracidium develops into an elongate sac, the mother sporocyst, which is usually located in the head-foot region of the snail. Daughter sporocysts are formed in the mothers from a small group of germinal cells carried in the miracidium. Production of the daughters is accomplished, at least in part, by simple multiplication of the germinal cells and the eventual development from them of embryo daughters. In *T. stagnicolae* this process is supplemented by polyembryony by means of germinal masses. In all three species the daughters migrate when very small, and at that time they usually contain only single germinal cells in the lumen although those of *S. douthitti* may contain small cercarial embryos. Further development of the daughters occurs principally in the digestive gland where they become intimately associated with the snail tissue. Multiplication within the daughter sporocysts involves both a multiplication of the germinal cells and multiplication by means of germinal masses. In *S. mansoni* and *S. douthitti* most of the cercarial embryos arise directly from single germinal cells without intervention of germinal masses. In *T. stagnicolae* each of the germinal cells carried by the young daughter sporocyst probably develops into a germinal mass. All the cercariae are thus produced from germinal masses in that species. In all three species the cercariae leave the daughter sporocysts by way of a terminal birth pore.

SUMMARY

A study was made of the early developmental stages of *Schistosoma mansoni* in the snail host, *Australorbis glabratus.* The mother sporocyst of *S. mansoni* is an elongate, thin-walled sac which lies, tightly convoluted, usually near the external surface in the head-foot region of the snail. From 200 to 400 daughter sporocysts are produced within each mother sporocyst by multiplication of the cells of the germinal line carried by the miracidium. Each miracidium carries 50 to 100 germinal cells and these divide to produce 200 to 400 germinal cells. Each of these germi-

nal cells then gives rise to a daughter sporocyst embryo. No germinal masses were found in mother sporocysts. The daughter sporocysts leave the mother sporocyst and migrate to the digestive gland or, occasionally, to other organs of the snail. At the time of migration the daughter sporocyst is a small elongate sac containing 50 to 100 germinal cells in the lumen. After reaching the definitive location the daughter sporocysts elongate and enlarge and ultimately give rise to numerous cercarial embryos. Most of the cercarial embryos arise from separate germinal cells which result from division of the germinal cells carried by the migrating daughter sporocysts. However, some of the germinal cells give rise to germinal masses which in turn produce cercarial embryos by a special form of polyembryony. The cercariae leave the sporocysts through a terminal birth pore. The early developmental stages of *Schistosoma mansoni* are compared with those previously described for *Trichobilharzia stagnicolae* and *Schistosomatium douthitti*. In all essential characteristics the early development of these three species is closely similar.

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POST-CERCARIAL DEVELOPMENT OF *SCHISTOSOMA MANSONI* IN THE RABBIT AND HAMSTER AFTER INTRAPERITONEAL AND PERCUTANEOUS INFECTION*

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It is agreed by most authorities (Miyagawa and Takemoto 1921, Faust and Meleney 1924, Faust, Jones and Hoffman 1934) that the normal route of migration of the schistosomes of man which gain access to the mammalian host by penetration of the skin is as follows. They enter capillaries or venules of the peripheral circulation, are carried in the venous blood stream through the heart to the lungs, work their way through the lung capillaries, are carried in the arterial blood stream to the mesenteric capillaries from which they enter the portal circulation, are carried to the liver and finally, as they mature, return by active movement against the portal blood stream into the mesenteric veins.

When cercariae are injected into the peritoneal cavity in experimental infections they are suddenly placed in a new environment which may be different in its reaction to their presence from that in the skin and subcutaneous tissue which they enter gradually by skin penetration. Furthermore, the early part of the migration must be somewhat different. The cercariae either must penetrate the parietal peritoneum and enter blood vessels from which they will be carried to the lungs, or they must penetrate the visceral peritoneum and enter the blood vessels of the portal system from which they will be carried to the liver.

While conducting experiments to determine the value of various experimental animals as hosts for *Schistosoma mansoni* a great disparity became apparent between worm yields from rabbits infected intraperitoneally and those infected percutaneously (Moore, Yolles and Meleney, 1949). Six to eight weeks after the intraperitoneal injection of 500 to 15,000 cercariae only 0.3% were recovered as worms. After percutaneous infections with the same number of cercariae 11.8% were recovered as worms. In mice and hamsters, on the other hand, there was little difference in the infections which resulted from the two methods of exposure. This raised the question of the fate of the cercariae after intraperitoneal injection in the rabbit. Among the possibilities are (1) that the worms were destroyed in the peritoneal cavity, (2) that they were destroyed in the tissues lining the peritoneal cavity, (3) that they were unable to gain entrance to venules through which they could reach the lungs, (4) that those which did gain entrance to blood vessels had been unfavorably affected by their stay in the peritoneal cavity so that they were unable to complete their migration to the portal circulation and to develop to maturity. This paper reports experiments performed to test these possibilities.

METHODS

Two series of rabbits were exposed to the cercariae of *S. mansoni,* one intraperitoneally, the other percutaneously, via ear immersion. One rabbit from each

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series was sacrificed daily after exposure. The young worms were recovered by irrigation of the peritoneal cavity, by perfusion of various parts of the circulatory system, and by mincing certain organs; and their stage of development and position in the host were noted. In addition two series of hamsters were used for comparison, one infected intraperitoneally, the other percutaneously by immersion of the shaved animal.

Infections. Suspensions of cercariae were secured from laboratory reared and infected *Australorbis glabratus*. The number of cercariae per ml of suspension was determined by sample counts of known volumes of suspension according to the technique described by Moore, Yolles and Meleney (1948). Large numbers of cercariae were used so that the number of young worms recovered would be sufficient for comparative study. It was planned to use 10,000 cercariae for each rabbit and 1,000 for each hamster but, since at the time of these experiments our supply of infected *Australorbis* was low and the shedding of cercariae was erratic, it was impossible to adhere rigidly to this plan.

For percutaneous infections of hamsters the posterior third of the animal was shaved and the animal was immersed for about one hour to a depth of about 2 inches in water containing the cercariae. Rabbits were exposed by immersing the ears in a coplin jar containing the suspension. The water in the jar was examined periodically until living cercariae could no longer be found.

For intraperitoneal injection of cercariae an 18-gauge needle was used for rabbits, and a 20-gauge needle for hamsters.

Worm Recovery. The following autopsy procedure was used. Hamsters were killed by ether. Rabbits were anesthetized and exsanguinated either by decapitation or by cannulation of the carotid artery. The blood was collected in tap water. This laked blood was examined for young worms but none were ever found. Both rabbits and hamsters were then skinned and washed with tap water to prevent hair from contaminating fluid which was to be examined microscopically. Next the peritoneal cavity was opened by an incision along that part of the abdomen where the abdominal cavity was greatest in depth, viz. from a point just caudad to the umbilical scar to the xyphoid process. Hemostats were used to hold up the cut edges of the abdominal wall. The cavity was filled by washing the walls and organs with a stream of citrated physiological saline under pressure. Care was used in washing the liver and spleen since too much pressure would rupture these delicate organs. When the cavity was full the fluid was aspirated using a hypodermic syringe without a needle. This procedure was repeated twice and the fluid from all three washings was collected in a flask or beaker.

When this was completed the heart and lungs were removed together in the following manner. The trachea was clamped and severed caudad to the larynx. Then the following vessels were severed after the application of double ligatures: the ascending aorta, the superior vena cava at its entrance to the right atrium, the inferior vena cava above the diaphragm. These are the essential separations. In addition double ligation and severing of the left innominate vein minimized bleeding when the lungs and heart were removed. After carefully removing all pleural attachments and separating the trachea from the esophagus, the lungs and heart were removed and placed in a separate container.

The right atrium and ventricle were opened and rinsed from atrium to ventricle with citrated saline under pressure. The fluid was collected in an appropriate container. The same procedure was followed with the left heart and the fluid collected separately.

The lungs were then perfused. The perfusion apparatus has been described in a previous publication (Yolles *et al* 1947). The lungs were perfused in three directions; namely, from pulmonary vein to pulmonary artery, from pulmonary artery to pulmonary vein, and from either pulmonary artery or vein to trachea.

For perfusion from vein to artery a cannula attached to a perfusion line was inserted into the opening in the left atrium directed into the pulmonary vein and the perfusing fluid was collected from the openings in the right heart. For perfusion from artery to vein the cannula was inserted through the right ventricle into the pulmonary artery and the fluid was collected from the openings in the left heart. The same procedures were used in both hamsters and rabbits, varying only in the size of the cannula used. During both of these perfusions the clamp on the trachea remained in place. To recover worms which may have escaped from capillaries into alveoli the clamp on the trachea was removed, an artery clamp was applied to the pulmonary artery or vein and perfusion was performed via the vein or artery. During any of these perfusions the lungs expand to almost three times their normal size. It was found that for the maximum recovery of worms the perfusion must be continued for one to two minutes after the perfusate is no longer tinged with blood. After the final perfusion the lungs were cut into small pieces and were allowed to stand in perfusing fluid. Later the fluid was examined for worms.

The mesenteric veins were perfused while the liver was in situ. The liver was protected against loss of intrahepatic fluid by the ligature already placed on the inferior vena cava above the diaphragm, by another ligature placed on the inferior vena cava between the junction of right renal vein and the liver, and by a ligature placed on the portal vein. Next the descending thoracic aorta was ligated. A cannula attached to a clamped pressure line was introduced into the aorta below its ligature. Another cannula attached to a length of rubber tubing was tied into the portal vein pointing toward the intestines, and its free end was placed in a collecting vessel. Care was taken to fill all tubing and cannulae with fluid before insertion into blood vessels in order to avoid the injection of air or its contact with blood before the blood became mixed with the anticoagulant. A detailed description of this procedure has been published (Yolles *et al* 1947). Instead of observing the described modifications of this technique for small animals we used the same technique for hamsters as for rabbits in order to minimize differences in recovery of worms. The lower extremities were perfused at the same time as were the mesenteric vessels. For this purpose a third cannula was used in the abdominal vena cava. A ligature was first applied to the vena cava about 4 cm above its origin in the rabbit and about 2 cm above its origin in the hamster. The cannula, prepared in the same manner as for the portal vein, was tied into the vena cava just below the ligature. The rubber tubing from this cannula led to a separate, appropriately labeled beaker or flask (fig. 1).

When the perfusion of the lower extremities and the mesenteric vessels was completed, the liver was removed and perfused. A cannula, attached to a pressure perfusion line was inserted into the hepatic vein and the liver perfusate was collected

from the portal vein. See Yolles *et al* (1947) for details of this technique. After perfusion the liver was cut into thin slices and placed in citrated saline.

In animals exposed intraperitoneally the retroperitoneal lymph nodes were removed. In hamsters exposed percutaneously the popliteal lymph nodes were removed since the animal's hind quarters were immersed. In rabbits exposed percutaneously by immersing the ears the cervical lymph nodes were removed. Occasional checks were made, such as examining the retroperitoneal lymph nodes of animals infected percutaneously. All excised lymph nodes were minced and allowed to stand in citrated saline.

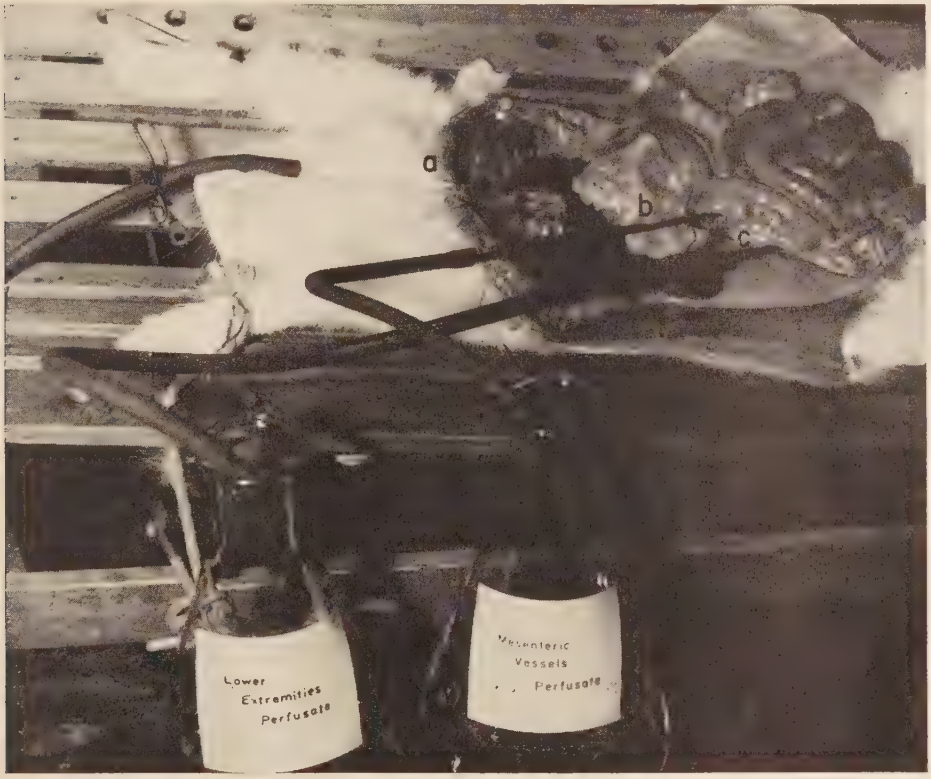

FIG. 1. Photograph of rabbit showing simultaneous perfusion of lower extremities and mesenteric vessels from the thoracic aorta. a. Cannula in thoracic aorta. b. Cannula in portal vein. c. Cannula in inferior vena cava.

The ears of rabbits exposed percutaneously and sacrificed on the first, second, fifth and ninth days were examined for worms by mincing portions from which the hair had been clipped. The minced tissue was allowed to stand in citrated saline. This method of worm recovery was, of course, inadequate for determining the total number of worms present in the tissue. It was used only to obtain some idea of the persistence of worms in this locality.

One hamster and one rabbit injected intraperitoneally were autopsied immediately after infection and the peritoneal cavities were washed to determine the number of cercariae which could be recovered. About 15 minutes elapsed between the time of injection of the cercariae and the completion of the washing of the cavity.

In all cases where minced tissue was placed in citrated saline it was allowed to stand for at least 1 hour before examination. Perfusates were centrifuged prior to examination. Fluid containing minced tissue was strained through a wire sieve, the tissue was washed and the fluid centrifuged. Since early stages of the worms are difficult to find and since the sediments were quite opaque, usually only a drop or two of the sediment was placed in a watch glass at a time. This was diluted with saline and examined through a dissecting microscope. As the worms were found they were removed with a fine capillary pipette and the stage of development and location in the host noted.

In classifying the stages of development of schistosomulae we used the Greek letters representing the 24 stages described by Faust and Meleney (1924) for *S. japonicum* and later adapted by Faust, Jones and Hoffman (1934) to *S. mansoni.*

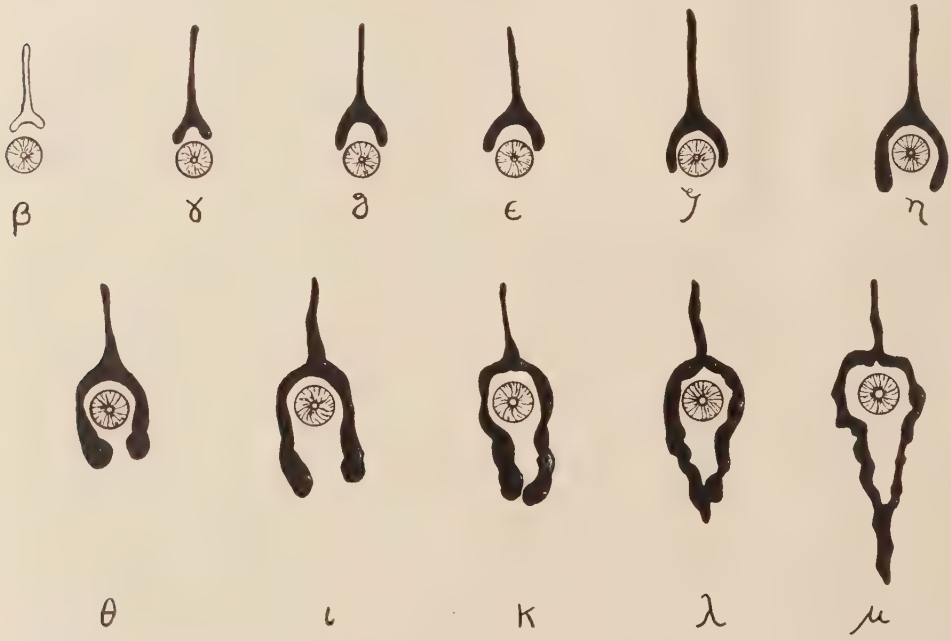

FIG. 2. Diagram of the development of the caeca of *Schistosoma mansoni* from the beta through the mu stage.

This classification is based upon the eighteen stages for *S. japonicum* described by Cort (1921), to which Faust and Meleney added 6 stages not described by him.

Since there are certain discrepancies in the designation of stages in the development of the worms between Faust and Meleney and Faust, Jones and Hoffman, we have used the criteria given by the latter authors in their diagrammatic presentation of these stages in *S. mansoni.* These criteria are relatively easy to follow in the early stages of development, but are difficult in the later stages. Since the length of mature worms varies in different hosts, the determination of maturity in male worms must be rather arbitrary. In the case of females our criterion for complete maturity was the presence of an egg in the ootype or uterus.

It was decided to group the alpha and beta stages together because it was felt that it would not affect the interpretation of the results and because the examination

of all these minute worms microscopically for distinguishing characters would be too time consuming. Beyond the beta stage the degree of caecal growth was used as the major criterion for determining the stage. In the beta stage the two minute caecal pouches lie just anterior to the ventral sucker. In the stages which follow the pouches grow around the acetabulum and at the lambda stage join at a distance posterior to it. Subsequent development consists of the lengthening of this joined segment posterior to the acetabulum (fig. 2) and of the development of the gynecophoral canal and of the testes in the male and the genital organs in the female.

RESULTS

Table 1 presents a summary of the percentage of cercariae recovered as worms from each series of animals on each day after infection on which animals were sacrificed. Tables 2, 3, 4 and 5 present the number and percentage of worms recovered from each locality in the host on each day on which animals were sacrificed. It is necessary to present both numbers and percentages because neither gives adequate information by itself. The stages of development of the worms from each location in each animal were determined and tabulated, but it is not practical to present all these tabulations. Summaries for each animal are presented in the text, and the stages of development of young worms recovered from each location in each series during the first nine days after infection are presented in Table 6.

Rabbit Intraperitoneal Series. Animals were examined at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 28, 42 and 63 days after infection. All the animals in this series received between 8,000 and 10,000 cercariae except the 63 day animal which received only 3,500 (Table 1). The worm recovery from the rabbit autopsied immediately after the injection of cercariae was only 54% of the number of cercariae injected. It seems probable that the failure to recover a higher percentage was due, at least partly, to attachment of cercariae to the peritoneum, and their partial penetration during the interval between the injection and the irrigation of the cavity. The recovery from the remainder of the series was very low, ranging from 0.2 to 4.0%. It was highest on the first and second days after infection when the worms were recovered almost exclusively from the peritoneal cavity (Table 2). Worms were recovered from this locality in every animal in the series. During the first 6 days after infection these worms represented over 80% of the total recovery. There was definite evidence of development of these worms, but this development was slow and often abnormal. From one day through 2 weeks of infection none of the schistosomulae recovered from the peritoneal cavity had advanced beyond the gamma stage, and the beta stage predominated. In the four-weeks infection, although the beta stage still predominated, one eta and one kappa stage were found. In the six-weeks infection there were 3 beta, 1 gamma, 2 theta, 2 iota, 2 kappa and 1 mu forms. Although these forms contained neither erythrocytes nor hematin the caeca were fairly well filled with a white opaque material which may have been mesothelial cells and cellular debris. In addition there was one dead worm to which no stage could be ascribed, and what appeared to be a twinned form or 2 organisms each about the size of a delta stage adhering at their posterior ends. No internal development was discernable. In the nine-weeks infection the peritoneal cavity contained two worms, one small and one large and bloated. The internal development in both, however,

TABLE 1.—*Cercariae Recovered as Worms in Each Series by Days after Infection*

Animal	Series		Days after Infection														
			0**	1	2	3	4	5	6	7	8	9	10	14	21	28	42	63
Rabbit	Intraperitoneal	Cercariae	12,300	8,500	8,000	8,500	8,500	10,000	10,000	8,500	10,000	8,500	.....	10,000	.....	10,000	10,000	3,500
		Worms Recov.	6,705	312	305	152	16	83	267	70	122	128	.....	83	.....	78	60	19
		% Recovery	54.5	3.7	3.8	1.8	0.2	0.8	2.7	0.8	1.2	1.5	.....	0.8	.....	0.8	0.6	0.5
	Percutaneous	Cercariae	.....	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	.....	10,000	10,000	10,000
		Worms Recov.	.....	160*	8*	14	1,350	1,301*	1,117	473	137	320*	555	715	.....	386	3,827	4,629
		% Recovery	.....	1.6*	0.1*	0.14	13.5	13.0*	11.2	4.7	1.4	3.2*	5.6	7.2	.....	3.9	38.3	46.3
Hamster	Intraperitoneal	Cercariae	1,000	1,000	1,000	1,000	500	500	1,000	1,000	900	500	600	.....	.....	.....	.....	.....
		Worms Recov.	587	93	60	84	63	126	134	63	129	122	145	.....	.....	.....	.....	.....
		% Recovery	58.7	9.3	6.0	8.4	12.6	25.2	13.4	6.3	14.3	24.4	24.2	.....	.....	.....	.....	.....
	Percutaneous	Cercariae	.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	.....	1,000	1,000	1,000	.....
		Worms Recov.	.....	0	0	2	54	255	25	287	101	33	132	.....	154	201	512	.....
		% Recovery	.....	0.0	0.0	0.2	5.4	25.5	2.5	28.7	10.1	3.3	13.2	.....	15.4	20.1	51.2	.....

** Worms recovered from peritoneal cavity within 15 minutes after injection of cercariae.
* Ear tissue also examined.

had not proceeded beyond the epsilon or zeta stage. Neither form contained hematin.

On the third day after infection there was evidence of migration out of the peritoneal cavity. One schistosomula was recovered from the retroperitoneal lymph nodes and two from the lungs. Worms were also recovered from the retroperitoneal lymph nodes on the seventh, eighth and ninth days. None had developed beyond the beta stage. It is probable that they had reached this location from the peritoneal cavity via the lymphatic vessels, and that they would ultimately die in the lymph nodes unless they could escape through efferent lymphatics.

Worms recovered from the right and left heart either must have been passing through in the blood stream to or from the lungs, or must have attached themselves to the endocardium, probably at some points outside the main course of the blood

TABLE 2.—*Rabbit Intraperitoneal Series. Worm Recovery by Location*

Cercariae	12,300	8,500	8,000	8,500	8,500	10,000	10,000	8,500	10,000	8,500	10,000	10,000	10,000	3,500
Days after Infection	0	1	2	3	4	5	6	7	8	9	14	28	42	63
Location	*Number of Worms Recovered by Location*													
Peritoneal Cavity	6,705	312	305	149	15	69	229	40	53	88	16	6	13	2
Lymph Nodes	...	0	0	1	0	0	0	4	3	2	0	0	0	0
Right Heart	...	...	...	...	...	3	9	3	0	15	0	0	0	0
Lungs	...	0	0	2	1	11	28	23	53	16	17	3	0	1
Left Heart	...	...	...	...	...	0	1	0	0	3	0	0	0	0
Lower Extremities	...	0	0	0	0	0	0	0	1	0	0	0	0	2
Mesenteric Veins	...	0	0	0	0	0	0	0	1	2	10	12	15	0
Liver	...	0	0	0	0	0	0	0	11	2	40	57	32	14
Total	6,705	312	305	152	16	83	267	70	122	128	83	78	60	19
Extraperitoneal	...	0	0	3	1	14	38	30	69	40	67	72	47	17
	Percentage of Total Worms Recovered by Location													
Peritoneal Cavity	100	100	100	98	94	83	86	57	43	68	19	8	22	11
Lymph Nodes	...	0	0	1	0	0	0	6	2	2	0	0	0	0
Right Heart	...	...	...	...	...	4	3	4	0	12	0	0	0	0
Lungs	...	0	0	1	6	13	11	33	43	12	21	4	0	5
Left Heart	...	...	...	...	...	0	(0.4)	0	0	2	0	0	0	0
Lower Extremities	...	0	0	0	0	0	0	0	1	0	0	0	0	11
Mesenteric Veins	...	0	0	0	0	0	0	0	1	2	12	15	25	0
Liver	...	0	0	0	0	0	0	0	10	2	48	73	53	73
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Extraperitoneal	...	0	0	2	6	17	14	43	57	32	81	92	78	89

stream. In this series of rabbits the heart was not perfused until the fifth day after infection when 3 worms were recovered from the right heart. Since worms were found in the lungs on the third day they must have passed through the right heart by that time. They were last found in the right heart on the ninth day indicating that passage into the lungs occurred at least until that day. The number of worms in the lungs increased until a peak of 53 was reached on the eighth day. This was followed by a decline although one beta stage was recovered from the lungs 63 days after infection. From the left heart one worm was recovered on the sixth day indicating escape from the lungs by that time. The only other worms recovered from the left heart were 3 on the ninth day. The recovery of fewer worms from the left than from the right heart occurred in all four series of animals.

Very little development took place in the lungs during the first 10 days after

infection. The oldest stage found was delta. At 2 weeks, of a total of 17 worms, 9 were beta, 6 gamma, 1 epsilon and 1 theta. Some worms of the gamma stage and all worms beyond this stage contained hematin in the gut. At 4 weeks, of a total of 3 worms, 2 were gamma and one kappa. At 6 weeks the lungs were negative, and at 9 weeks one worm in the beta stage was recovered.

The only worms recovered from the lower extremities were one on the eighth day and two on the sixty-third day. Even the latter worms were in the beta stage, indicating that some worms may exist for a long period of time in locations unfavorable for development.

From the liver and mesenteric vessels worms were first recovered on the eighth day after infection. The number of worms from the mesenteric vessels increased slowly through the sixth week but at 9 weeks no worms were recovered from this location. The worm recovery from the liver increased more rapidly and from two weeks onward this was the location from which most of the worms were recovered. On the eighth and ninth days no forms beyond the delta stage were found there. At 2 weeks the earliest stage was epsilon, the most numerous was theta and the most advanced was lambda. At 4 weeks the earliest stage was lambda, and the most advanced stage as well as the predominating stage was phi. At 6 weeks a few earlier stages (iota, theta and kappa) were found. Many of the worms were approaching maturity (phi stage) but none were mature. At 9 weeks one beta stage, one mu and two rho were found, and the remainder were in the phi stage.

Apparently worms recovered from the mesenteric vessels on the eighth and ninth days had just arrived there since only beta and gamma stages were recovered. On the fourteenth day the most advanced stage was lambda, the predominating stage was theta and the least developed was beta. On the twenty-eighth day the most advanced form was tau, the least developed was kappa and intermediate forms were found in about equal numbers. On the forty-second day the most advanced and the most numerous stage was chi and only one as early as the theta stage was found. At nine weeks no worms were found in the mesenteric vessels.

Remarks. The picture of migration and development of worms in the rabbit after intraperitoneal infection is as follows. Only 3.7% of the schistosomulae were recovered from the peritoneal cavity after one day, but some remained there and underwent a moderate amount of development. By the third day a few had reached the retroperitoneal lymph nodes and the lungs but none apparently reached the liver by direct penetration into the portal circulation. Accumulation in the lungs reached a peak, though a very low one, on the eighth day. Meanwhile migration out of the lung was evident on the sixth day from the finding of worms in the left heart. They began to enter the portal system by the eighth day and accumulated there slowly until at least the twenty-eighth day by which time practically all had left the lungs. Their development in the portal blood was slow, no fully mature worms being found even after nine weeks, and comparatively few migrated back from the liver to the mesenteric veins.

Remarkable about this series are: (1) the low worm recovery throughout, (2) the apparent high initial mortality of worms in the peritoneal cavity, (3) the persistence of some of the worms in this location for at least 9 weeks, (4) their development without portal blood, (5) the abnormal development of some of these worms,

and (6) the slow development of all worms. Also worthy of note is the persistence of young worms in the lungs through the period studied. For the first ten days the worms in the lungs showed no development beyond the delta stage. At 2 weeks and 4 weeks a few more advanced stages were found, the most advanced being the kappa. Although these latter forms may have developed in the lungs without leaving this location it is possible that they had migrated to the portal system, had undergone further development there, and were later carried back to the lungs.

Rabbit Percutaneous Series. Animals were examined daily from 1 through 10 days and at 14, 28, 42 and 63 days after exposure to 10,000 cercariae. Except for the first three days the percentage of worms recovered greatly exceeded that of the intraperitoneal series (Table 1). The greatest worm recovery was at 9 weeks after infection (46.3%), whereas in the intraperitoneal series the yield at this time was one of the lowest (0.5%).

TABLE 3.—*Rabbit Percutaneous Series. Worm Recovery by Location*

Cercariae	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000
Days after Infection	1	2	3	4	5	6	7	8	9	10	14	28	42	63
Location	*Number of Worms Recovered by Location*													
Ears	159	7	...	...	5	...	...	...	5	...	...	...	...	...
Lymph Nodes	1	...	...	...	...	...	...	...	...	...	...	...	...	...
Right Heart	0	1	...	58	322	19	2	...	7	14	0	0	0	0
Lungs	0	0	14	1,252	960	1,060	457	98	167	444	112	0	0	0
Left Heart	0	0	...	2	14	30	8	...	80	25	2	0	0	0
Lower Extremities	0	0	0	23	0	1	0	9	0	33	0	0	0	0
Mesenteric Veins	0	0	0	0	0	0	0	3	7	8	35	65	973	2,828
Liver	0	0	0	0	0	1	6	27	54	31	566	321	2,854	1,801
Total	160	8	14	1,335	1,301	1,111	473	137	320	555	715	386	3,827	4,629
	Percentage of Total Worms Recovered by Location													
Ears	99	88	...	...	(0.4)	...	...	2	...	...	...	...	...	...
Lymph Nodes	1	...	...	...	...	...	...	...	...	...	...	...	...	...
Right Heart	0	12	...	4	25	2	(0.4)	...	2	2	0	0	0	0
Lungs	0	0	100	94	74	95	97	71	52	80	16	0	0	0
Left Heart	0	0	...	(0.2)	1	3	2	...	25	5	(0.3)	0	0	0
Lower Extremities	0	0	0	2	0	(0.1)	(0.2)	7	0	6	0	0	0	0
Mesenteric Veins	0	0	0	0	0	0	0	2	2	1	5	17	25	61
Liver	0	0	0	0	0	(0.1)	1	20	17	6	79	83	75	39
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100

The animal autopsied one day after exposure yielded no schistosomulae. Because of this another animal was exposed and was autopsied the following day. One ear of this animal was minced and steeped in saline. When the tissue was removed and the sediment examined 159 worms were recovered (Table 3). In addition one worm was recovered from the cervical lymph nodes. On the second day 7 worms were recovered from ear tissue and one from the right heart. The latter is the first indication of migration through the venous circulation toward the lungs. On the third day the heart and ears were not examined. All the worms found were in the lungs and were in the alpha and beta stages. On the fourth day there was a great increase in the total number of worms recovered. The right heart contained 58 worms and the lungs 1,252. Migration out of the lungs had already begun as evidenced by the recovery of 2 worms from the left heart. The recovery of 23 worms from the lower extremities indicates that they were being distributed into peripheral systemic vessels. Maximum recovery from the right heart occurred on

the fifth day when 322 were found. The number in the lungs remained high (960). Migration out of the lungs increased (14 worms in left heart). The number of worms recovered from the right heart decreased rapidly after the fifth day. On the fourteenth day and thereafter this location was negative. Worm recovery from the lungs remained high and accounted for most of the worms recovered through the tenth day. At 2 weeks the recovery from the lungs dropped to 16%. At 4 weeks, 6 weeks and 9 weeks this location was negative. Peak recovery from the left heart occurred on the ninth day and the left heart remained positive through the fourteenth.

Worms were first recovered from the liver on the sixth day after infection when a single form in the gamma stage was found. Numbers in the liver increased steadily. In animals autopsied at 2, 4 and 6 weeks, more than 70% of the worms recovered were from the liver, the maximum number being 2,854. The percentage recovery from the liver finally decreased while the percentage recovery from the mesenteric vessels increased, indicating migration of maturing worms backward from the liver toward the intestine. Worms were first recovered from the mesenteric vessels on the eighth day. They increased until on the sixty-third day 2,828 worms, 61% of the total, were recovered from this location.

Although worms were recovered from the ears as late as 9 days after exposure, all of them were in the alpha or beta stage. Since the ears were not examined after that day it is not known how much longer worms remained there.

From the right heart all the worms recovered were alpha, beta and gamma forms except for one epsilon form found on the sixth day. In the lungs the beta stage predominated throughout the entire series. Gamma forms first appeared on the fifth day. On the seventh through the tenth day a few epsilon forms were found. On the fourteenth day in addition to beta, gamma, delta and epsilon forms in about the same proportion as before, there were two zeta forms and one kappa. Thereafter the lungs were negative. Only alpha, beta and gamma forms were recovered from the left heart.

In the liver, only beta and gamma forms were found until the eighth day when delta forms appeared. They remained the most advanced form on the ninth day. On the tenth the most advanced form was eta, the least developed was gamma and most of the worms were in the delta stage. On the fourteenth day the lambda stage was the most advanced; most of the worms were in the iota stage but a considerable number of beta forms were still present. These were probably recent arrivals from the lungs. On the twenty-eighth day the worms recovered from the liver ranged from the iota through the rho stage. On the forty-second day they ranged from the lambda through the phi stage. Although there were at least 32 pairs in copula the worms were small and the females did not contain an egg in the oötype. On the sixty-third day the earliest stage was still lambda. Most of the worms were approaching maturity and some were fully mature with an egg in the oötype of the female.

Development of worms in the mesenteric vessels was similar to that in the liver on the eighth through the fourteenth day. On the twenty-eighth day the range was from iota through tau with most of the worms in the more advanced part of the range. Although lambda stages were still found on the forty-second and the sixty-third day most of the worms were approaching maturity and a few were mature on the forty-second day, and most were mature on the sixty-third day.

Remarks. On the first three days following percutaneous exposure of rabbits the worm recovery was very low. This may be because of a slow migration away from the ears where some remained at least through the ninth day after exposure. This together with the recovery of large numbers of very young worms from the lungs from the fourth through the fourteenth day, indicates that they are probably capable of entering blood vessels in the ears from which they can be carried to the lungs for several days and possibly as long as fourteen days after penetrating the skin. The recovery of a schistosomula from the right heart on the second day is the first indication of migration toward the lungs. This migration through the right heart apparently reached its peak on the fifth day and continued through at least the fourteenth day. The recovery of 322 worms from the right heart on the fifth day deserves special comment. The possible explanations for the recovery of so many worms from this location seem to be either that they were attached to the endocardium at points where the blood was not in rapid passage through the chambers, or that dilatation of the peripheral capillaries of the body at the time the rabbit was killed had released many worms which were able to reach the heart before the heart stopped beating. The accumulation of worms in the lungs was greatest between the fourth and the tenth day. Exodus from the lungs started by the fourth day and continued until some time between the fourteenth and twenty-eighth day as evidenced by the recovery of worms from the lungs and left heart on the fourteenth day but not on the twenty-eighth day. No worms beyond the epsilon stage were found in the lungs except that two zeta and one kappa were recovered on the fourteenth day. Since at this time worms were established in the hepatic and mesenteric vessels it is not known whether these three organisms developed in the lungs or were carried back to the lungs via either portal systemic anastomoses or the central veins of the liver.

The next capillary barriers for the young worms beyond the lungs are those of the periphery of the body and the mesenteric system. This is evidenced by the recovery of young worms from the lower extremities by the fourth day and intermittently thereafter through the tenth day. Although they were not recovered from the mesenteric vessels until the eighth day, their presence in the liver by the sixth day indicates that some had already passed through the mesenteric capillaries. The fact that the number of worms recovered from the liver increased slowly while the number in the lungs was decreasing more rapidly suggests that they were being distributed to all parts of the body, and that relatively few reached the mesenteric circulation at any one time. Between the tenth and the fourteenth day, however, there was a marked increase of worms recovered from the liver, and parallel with this a marked decrease of worms from the lungs. Again there was a great increase of worms recovered from the liver between the twenty-eighth and forty-second day, after they had disappeared from the lungs. This is difficult to explain unless it is because many young worms were held so tightly in the portal radicals of the liver that they could not be recovered by perfusion or slicing of the liver, whereas they were easier to recover after they had returned during maturation to the larger portal branches. The increase in worms recovered from the mesenteric vessels parallels that from the liver, except that it was more gradual, and that the number ultimately exceeded that from the liver, due to the migration of the more mature worms back into the mesenteric veins. It is interesting that worms of as mature development

as the lambda stage were recovered from the mesenteric vessels as early as the fourteenth day. Although the general conception of the route of migration is that young worms which pass through the mesenteric capillaries are carried into the liver and later work their way back against the blood stream into the mesenteric veins, it seems possible that these worms may have remained in the mesenteric venules and undergone development there.

Hamster Intraperitoneal Series. Animals were examined immediately after injection and daily through the first 10 days. This was considered sufficient time in which to compare the early migration and development with those in the rabbit. The hamsters autopsied on days 1, 2, 3, 6 and 7 after infection received 1,000 cercariae; that on day 8 received 900, that on day 10 received 600 and those on days 4, 5 and 9 received 500.

Autopsy immediately after infection yielded a recovery of 59% (Table 1).

TABLE 4.—*Hamster Intraperitoneal Series. Worm Recovery by Location*

Cercariae	1000	1000	1000	1000	500	500	1000	1000	900	500	600
Days after Infection	0	1	2	3	4	5	6	7	8	9	10
Location					*Number of Worms Recovered by Location*						
Peritoneal Cavity	587	83	58	74	19	72	12	26	51	59	26
Lymph Nodes	...	0	2	0	0	2	0	0	0	0	0
Right Heart	...	...	...	9	...	...	13	...	...	...	...
Lungs	...	0	0	1	30	35	91	10	1	0	0
Left Heart	...	...	...	0	...	...	8	...	...	...	...
Lower Extremities	...	0	0	0	0	0	3	0	0	0	0
Mesenteric Veins	...	0	0	0	6	11	0	17	38	10	10
Liver	...	10	0	0	8	6	7	10	39	53	109
Total	587	93	60	84	63	126	134	63	129	122	145
Extraperitoneal	...	10	2	10	44	54	122	37	78	63	119
					Percentage of Total Worms Recovered by Location						
Peritoneal Cavity	100	89	98	88	30	57	9	41	40	49	18
Lymph Nodes	...	0	2	0	0	1	0	0	0	0	0
Right Heart	...	...	...	11	...	...	10	...	...	...	...
Lungs	...	0	0	1	48	28	68	16	1	0	0
Left Heart	...	...	...	0	...	...	6	...	...	...	...
Lower Extremities	...	0	0	0	0	0	2	0	0	0	0
Mesenteric Veins	...	0	0	0	9	9	0	27	29	8	7
Liver	...	11	0	0	13	5	5	16	30	43	75
Total	100	100	100	100	100	100	100	100	100	100	100
Extraperitoneal	...	11	2	12	70	43	91	59	60	51	82

About 15 minutes elapsed between the time of injection of cercariae and the completion of the washing of the peritoneal cavity, during which time some of the cercariae may have penetrated the peritoneum. The worm recovery from the rest of the animals in this series ranged from 6% to 25%. Excluding the first 3 days when only a small percentage of those recovered had left the peritoneal cavity, the mean worm recovery was 17%. Schistosomulae were recovered from the peritoneal cavity in all of the animals examined (Table 4). Ten worms were recovered from the liver on the first day. It is possible that these worms were clinging to the outside of the liver, or had penetrated directly from the peritoneal cavity. It is also possible that they had penetrated the visceral peritoneum and had entered the portal circulation. No other worms were found outside the peritoneal cavity on this day. On the second day the only positive location other than the peritoneal cavity was the retroperitoneal lymph nodes. Worms were found in this location on the fifth day

also. The right heart, which had not been examined on the second day, was positive on the third day. In addition a single schistosomula was found in the lungs. The lungs remained positive through the eighth day, the peak occurring on the sixth day. No worms were recovered from the liver on the second and third days. From the fourth day to the end of this series on the tenth day the liver was consistently positive and contained increasing numbers of worms. Worms were recovered from the mesenteric vessels on the fourth day and on all subsequent days except the sixth.

There was evidence of some development in the peritoneal cavity. During the first 5 days of infection only alpha and beta forms were present. On the sixth and seventh days a few gamma forms were found also. On the eighth, ninth and tenth days considerable elongation had taken place and the caeca were filled with a white opaque material. The schistosomulae recovered from the retroperitoneal lymph nodes were in the alpha or beta stage. Very little development took place in the lungs. No forms beyond the beta stage were found until the sixth day and no forms beyond delta were recovered from the lungs in the entire series. The schistosomulae found in the lower extremities on the sixth day were in the beta and gamma stages. Growth was rapid in the liver and mesenteric vessels. Although beta forms persisted through the tenth day, kappa stages were recovered from both liver and mesenteric vessels on the eighth day. Lambda stages appeared in the liver on the ninth day. No forms beyond the kappa were found in the mesenteric vessels.

Remarks. On the first, second and third days after infection the worm recovery was markedly lower than the recovery 15 minutes after injection of cercariae. The increase in worm recovery from the fourth day on indicates that the initial sharp decline was due at least in part to the escape of worms through the peritoneum and their delay in gaining entrance into blood vessels. The presence of schistosomulae in the liver one day after infection indicates escape from the peritoneal cavity as early as the first day unless these organisms were clinging to the outside of the liver. Either this is an unusual finding or when it occurs the worms do not remain in the liver, since no worms were recovered from this location on the second and third days.

Migration into the lungs had begun by the third day and reached its peak on the sixth day. Migration out of the lungs began by the fourth day, as indicated by the presence of worms in the mesenteric vessels and in the liver, and exodus from the lungs was complete by the eighth day. The schistosomulae developed rapidly in this series. This is probably due mainly to their early localization in the liver, but may be due also to the fact that the hamster is a very favorable host.

Hamster Percutaneous Series. Animals were examined at daily intervals from 1 through 10 days and at 21, 28 and 42 days after infection. All the animals in this series were exposed to 1000 cercariae. Excluding the first three days when few worms were recovered, the average worm recovery was 17.5%, ranging from 2.5% to 51.3% (Table 1).

No worms were recovered on the first and second days and only two (from the popliteal lymph nodes) on the third day (Table 5). On the fourth day worms were recovered from the right heart and the lungs. Recovery from the right heart reached a peak on the fifth day and worms were found there until the ninth day. The greatest number of worms in the lungs was found on the seventh day when 250, representing 87% of the total, were recovered. Thereafter the percentage of worms

recovered from the lungs declined. None was found there on the twenty-first and twenty-eighth days but on the forty-second day 9% of the worms recovered were from the lungs. This finding is commented upon below.

The left heart was first positive on the fifth day, reached a peak on the seventh and eighth days, and was negative after the tenth day. The lower extremity perfusate was positive on the eighth and tenth days for 2 and 5 worms respectively. Schistosomulae were first found in the liver on the eighth day (27 worms) and in the mesenteric vessels on the tenth day (16 worms). The worm recovery from these locations increased through the forty-second day when 294 worms were recovered from the liver and 173 from the mesenteric veins. From the ninth day to the end of the series the liver was the location of greatest worm recovery.

Worms in the right heart did not advance beyond the gamma stage except for two delta forms found on the ninth day. In the lungs beta and gamma forms pre-

TABLE 5.—*Hamster Percutaneous Series. Worm Recovery by Location*

Cercariae	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Days after Infection	1	2	3	4	5	6	7	8	9	10	21	28	42
Location	*Number of Worms Recovered by Location*												
Lymph Nodes	0	0	2	0	0	0	0	0	0	0	0	0	0
Right Heart	0	0	0	12	57	0	4	0	4	0	0	0	0
Lungs	0	0	0	42	189	23	250	55	8	37	0	0	45
Left Heart	0	0	0	0	9	2	33	17	0	13	0	0	0
Lower Extremities	0	0	0	0	0	0	0	2	0	5	0	0	0
Mesenteric Veins	0	0	0	0	0	0	0	0	0	16	18	37	173
Liver	0	0	0	0	0	0	0	27	21	61	136	164	294
Total	0	0	2	54	255	25	287	101	33*	132	154	201	512
	Percentage of Total Worms Recovered by Location												
Lymph Nodes	0	0	100	0	0	0	0	0	0	0	0	0	0
Right Heart	0	0	0	22	22	0	1	0	12	0	0	0	0
Lungs	0	0	0	78	74	92	87	54	24	28	0	0	9
Left Heart	0	0	0	0	4	8	12	17	0	10	0	0	0
Lower Extremities	0	0	0	0	0	0	0	2	0	4	0	0	0
Mesenteric Veins	0	0	0	0	0	0	0	0	0	12	12	18	34
Liver	0	0	0	0	0	0	0	27	64	46	88	82	57
Total	0	0	100	100	100	100	100	100	100	100	100	100	100

* Poor perfusion.

dominated from the first through the tenth day. Delta stages appeared on the fifth day and a few epsilon forms on the sixth day. On the twenty-first and twenty-eighth days this location was negative, but on the forty-second day 45 worms were found in the lungs. Most of these worms were fully mature though a few were in the phi stage. In the left heart the beta and gamma stages predominated and the most advanced stage was epsilon. Only early forms (through epsilon) were recovered from the lower extremities.

On the eighth day when worms were first found in the liver only beta and gamma forms were recovered. On the ninth day gamma forms still predominated and the most advanced forms were zeta. On the tenth day the range of development was from gamma through lambda and the predominating form was theta. On the twenty-first day the range was from theta through rho, with the predominating form lambda. On the twenty-eighth day the range was from theta through phi, most of the worms being in the latter stage. On the forty-second day most of the 294 worms in the liver were mature. In addition, one epsilon and 2 kappa stages and 25 worms between lambda and phi were found. The worms found in the

mesenteric vessels were similar in development to those in the liver except that in the older infections the early stages still present in the liver were absent from the mesenteric vessels.

TABLE 6.—*Distribution of Schistosomulae According to Location and Stage of Development during the First 9 Days after Infection*

Stage	Peritoneal Cavity	Lymph Nodes	Right Heart	Lungs	Left Heart	Lower Extremities	Mesenteric Veins	Liver
			A. *Rabbit Intraperitoneal Series*					
λ	..	..	..	..	..	..	..	..
κ	..	..	..	..	..	..	..	..
ι	..	..	..	..	..	..	..	..
θ	..	..	..	..	..	..	..	..
η	..	..	..	..	..	..	..	..
ζ	..	..	..	..	..	..	..	..
ε	..	..	..	..	..	..	..	..
∂	..	..	..	+	..	..	..	+
γ	+	..	+	+	..	..	+	+
α+β	+	+	+	+	+	+	+	+

Stage	Ears	Lymph Nodes	Right Heart	Lungs	Left Heart	Lower Extremities	Mesenteric Veins	Liver
			B. *Rabbit Percutaneous Series*					
λ	..	..	..	..	..	..	..	..
κ	..	..	..	..	..	..	..	..
ι	..	..	..	..	..	..	..	..
θ	..	..	..	..	..	..	..	..
η	..	..	..	..	..	..	..	..
ζ	..	..	..	..	..	..	..	..
ε	..	..	..	..	..	..	..	..
∂	..	..	..	+	..	+	+	+
γ	..	..	+	+	+	+	+	+
α+β	+	+	+	+	+	+	+	+

Stage	Peritoneal Cavity	Lymph Nodes	Right Heart	Lungs	Left Heart	Lower Extremities	Mesenteric Veins	Liver
			C. *Hamster Intraperitoneal Series*					
λ	..	..	..	..	..	..	..	+
κ	..	..	..	..	..	..	+	+
ι	..	..	..	..	..	..	+	+
θ	..	..	..	..	..	..	+	+
η	..	..	..	..	..	..	+	+
ζ	..	..	..	..	..	..	..	+
ε	..	..	..	..	..	..	+	+
∂	..	..	..	..	..	..	+	+
γ	+	..	+	+	+	+	+	+
α+β	+	+	+	+	+	+	+	+

Stage	Lymph Nodes	Right Heart	Lungs	Left Heart	Lower Extremities	Mesenteric Veins	Liver
		D. *Hamster Percutaneous Series*					
λ	..	..	..	..	..	..	..
κ	..	..	..	..	..	..	..
ι	..	..	..	..	..	..	..
θ	..	..	..	..	..	..	..
η	..	..	..	..	..	..	..
ζ	..	..	..	..	..	..	+
ε	..	..	+	+	+	..	..
∂	..	+	+	..	..	+	+
γ	..	+	+	+	+	+	+
α+β	+	+	+	+	+	..	+

Remarks. The inability to recover schistosomulae on the first three days after infection indicated that they were in regions other than those examined and suggested that they remained in the skin and subcutaneous tissues. To determine this, 1,000 cercariae were placed on the shaved abdomen of a hamster and four days later the animal was killed and the shaved skin removed, minced and allowed to stand in saline. Nine living worms were found in the fluid. All were still in the beta stage and probably still were capable of active penetration. It is possible, therefore, that schistosomulae in the hamster may remain in the tissue of the area of penetration for at least four days, and still be capable of entering the circulation.

Migration into the lungs began by the fourth day and probably ended on about the ninth day. Migration out of the lungs started on the fifth day and continued until some time between the tenth and the twenty-first day. The recovery of worms from the lower extremities on the eighth and tenth days indicates that there was a systemic distribution of worms from the lungs, and may explain the lag in time between the maximum recovery of worms from the lungs and the recovery of a similar number from the liver. The presence of worms in the lungs on the forty-second day after infection, when no worms had been recovered from this location on the twenty-first and twenty-eighth days can be explained on the basis of the large worm burden in this animal. There were 512 worms, most of them mature, recovered from this hamster. The physical limitations of vessel space may have caused some of them to migrate from the mesenteric vessels through anastomoses into the hemorrhoidal or esophageal veins thence to be carried back to the heart and lungs.

DISCUSSION

A comparison of the four series of animals shows that the intraperitoneal infection of the rabbit produced the poorest infection. The recovery of worms from the peritoneal cavity of these animals immediately after injection was almost the same, 54% from the rabbit and 58% from the hamster. The recovery from this location during the first three days after infection from rabbits was 3.7, 3.8 and 1.8%; from hamsters it was 8.3, 5.8 and 7.4%, indicating either that there was a higher mortality in the peritoneal cavity of the rabbit than of the hamster, or that a larger proportion of worms in the rabbit had successfully penetrated the surrounding tissues. In the rabbits of this series which were examined after the first three days there was no marked increase in the proportion of worms recovered from other parts of the body, whereas in the corresponding hamsters there was a considerable increase, indicating that the disappearance of worms from the peritoneal cavity of the rabbits was probably not due to their successful penetration into the blood vessels, but must have been due to a higher mortality in the peritoneal cavity or in the adjacent tissues. This is supported by the fact that in the percutaneous rabbit series worm recoveries from the longest infections were as high as 38.3 and 46.3%, indicating that the worms had not suffered a high mortality in penetration of the skin, or in passage through the blood vessels.

Faust, Jones and Hoffman (1934) report finding larvae in the skin of rats 22 hours after percutaneous exposure and that the skin was negative on examination at 42 hours and thereafter. Our data show that schistosomulae were recovered from the skin of hamsters for at least four days and from the ears of rabbits for at least nine days after exposure to cercariae. It is not known for how much longer living

worms can be recovered from these locations since these were the last days on which they were examined.

Development of schistosomulae was slower in the rabbits than in the hamsters and was fastest in the hamsters of the intraperitoneal series. Schistosomulae first appeared in the lungs on the third day in both rabbit series and in the hamster intraperitoneal series; on the fourth day in the hamster percutaneous series. The lungs became free of schistosomulae in the hamster intraperitoneal series on the ninth day, in the hamster percutaneous series between the tenth and the twenty-first, and in the rabbit percutaneous series between the fourteenth and the twenty-eighth day; while in the rabbit intraperitoneal series one young worm was recovered from the lungs nine weeks after infection.

In agreement with the work of Faust and Meleney (1924) on the migration of *S. japonicum* in mice and rabbits, and the work of Faust, Jones and Hoffman (1934) on migration of *S. mansoni* in rats we have no evidence of development in the lungs beyond the epsilon stage. Although we have found forms as mature as the lambda stage in the lungs early in the course of infection they were present at a time when these stages were also present in the portal system, and the possibility that such forms developed in the portal system and returned to the lungs must not be ignored. The presence of adult worms in the lungs in the hamster percutaneous series on the forty-second day after infection when they had been absent from this location on the twenty-first and twenty-eighth days is probably due to their late migration through anastomoses from the portal to the systemic venous circulation from which they were carried to the lungs.

Schistosomulae first appeared in the liver in the hamster intraperitoneal series on the fourth day except for the initial finding on the first day, in the rabbit percutaneous series on the sixth day, and in the hamster percutaneous series and rabbit intraperitoneal series on the eighth day. On the ninth day the most advanced stage in the hamster percutaneous series was zeta, whereas in the hamster intraperitoneal series development had advanced five stages more to lambda. In both rabbit series the most advanced stage on this day was delta. The hamster intraperitoneal series was not continued beyond the tenth day, but in the percutaneous series most of the worms developed to maturity between the fourth and sixth weeks. In the rabbit percutaneous series most of the worms became mature between the sixth and ninth weeks but in the rabbit intraperitoneal series no mature worms were found even at the end of nine weeks. Our observations on rabbits infected percutaneously confirm those of Faust, Jones and Hoffman (1934) with regard to the length of time required for *S. mansoni* to develop to maturity.

In conclusion, when the cercariae of *S. mansoni* are injected into the peritoneal cavity of the rabbit there is a high initial mortality and possibly a decrease in viability as evidenced by a very slow development to maturity. The nature of the condition in the peritoneal cavity which is unfavorable for the cercariae of *S. mansoni,* and its possible immunological significance, remain to be determined.

SUMMARY

1. The routes of migration and the stages of development of *Schistosoma mansoni* in various tissues and portions of the circulation of the rabbit and hamster following intraperitoneal and percutaneous infection are described.

2. It was found that in rabbits infected intraperitoneally a very small proportion of the worms were able to reach the lungs and portal circulation leading to the conclusion that there was a high mortality in the peritoneal cavity or in the adjacent tissues. This was in contrast to the finding that in rabbits following percutaneous infection, and in hamsters following either intraperitoneal or percutaneous infection a considerable proportion of the worms reached the lungs and portal circulation.

3. Schistosomulae were recovered from the skin or adjacent subcutaneous tissues of hamsters on the fourth day after percutaneous infection, and from the ears of rabbits as late as the ninth day.

4. The development of worms which reached the lungs and portal circulation in rabbits infected intraperitoneally was slower than in rabbits infected percutaneously suggesting that some of the "intraperitoneal worms" had suffered damage which was not apparent in worms following percutaneous infection.

5. The development of worms to maturity in rabbits infected percutaneously was slower than in hamsters similarly infected, indicating that the rabbit is a less favorable host than the hamster for *S. mansoni.*

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A NEW GENUS AND SPECIES OF MITE FROM THE NASAL CAVITY OF THE RING-BILLED GULL, (ACARINA, EPIDERMOPTIDAE).

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The material on which this paper is based was collected and prepared by Dr. Russell W. Strandtmann, and deposited in the United States National Museum to await identification. It was through the kindness of Dr. Edward Baker of this department that the author was given the opportunity to undertake the present investigation, and to whom thanks are due for his helpful criticism. The specimens were discovered in the nasal cavity of the ring-billed gull (*Larus delawarensis* Ord.) in Galveston, Texas between March 14 and 25, 1947. The following description has been compiled from 35 individuals,—including 11 females, 14 males, seven nymphs and three larvae. The material is in excellent condition and had been mounted in a modified Berlese mixture.

Turbinoptes, new genus

Body oval, sparsely haired. Dorsal shields present but weakly sclerotized. Legs of equal length; legs I and II of similar width but thicker than legs III and IV, which are also of equal width. Epimera I united in a V-form. Each tarsus very short, bearing a few setae, two claws and an ambulacrum. Sexual dimorphism present. Female: vulva transverse at level of coxa IV; posterior end of body rounded, with a pair of copulatory cones bearing terminal setae; copulatory suckers absent; ambulacra flask-shaped on long unsegmented peduncles. Male: ambulacra present only as mere stubs; body terminates as two lobes; copulatory suckers present; copulatory cones absent; genital aperture posterior to coxa IV; penis short and curved. GENOTYPE: *Turbinoptes strandtmanni* n. sp.

Turbinoptes strandtmanni, n. sp.

LARVA (Figs. 5–7). The entire body is delicately built with smaller and less well developed body components than in the nymph. The cuticle is finely striated along the anterior half of the dorsum. Leg IV is lacking. Leg III is directed posteriorly, genital aperture absent and ambulacra mere stumps,—all three features typical of the nymphal stage. Dorsum and general body plan very similar to the female. The ventral body setae consist of only the two epimeral, the single postero-lateral and a solitary anal pair; these anal setae representing the terminal setae of the adult, being 57 μ long and located on the simple copulatory cones. The setae of the legs are similarly reduced in number compared with the adult, there being no setae present on the trochanters, or femur and patella III; and they number five, five and three on tarsi I, II and III respectively. Size, 320–370 μ in length and 270–300 μ in width.

NYMPH (Figs. 13, 14). At first glance the nymph appears to be a replica of the female in which the genital aperture, the ambulacra and the reticulate shields of the female are absent. The entire body is more delicately built than in the adult. All seven nymphs examined are structurally similar to each other except in body size. Leg III, like leg IV, faces posteriorly, and the ambulacra (Fig. 14) consist as in the male of mere stumps, 15 μ in length. The two longest setae of the copulatory cones are 100 μ long. The legs possess fewer setae, in that none are present on trochanter IV and only six are present on tarsi I and II. The two smallest nymphs measure 381–384 μ by 300–336 μ; two others, 468–476 μ by 357 μ and the three largest are 492–550 μ in length and 370–402 μ in width,—one of which is in the process of molting into a female.

ADULT FEMALE. (Figs. 1, 8): *Body* oval, sparsely haired. Cuticle soft with fine parallel lines extending over the greater part of the body, but absent on its anterior quarter. These lines, however, are replaced by fine reticulate shields in the following areas,—dorsally, over the base of the capitulum and the coxae, and over a wide area of the posterior half of the body terminating in two concentrations, one on each side at the level of the copulatory cones: ven-

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trally, as two small regions lateral to the cones. Three small globular deposits are evident on the venter, one slightly to the left of the junction of epimera I, and the other two at the base of each epimeron II. Eyes lacking. A pair of small pores lies on the postero-lateral border of the capitulum. No suture is visible between the propodosoma and hystriosoma. The posterior extremity of the body is round and the anus is ventral to subterminal. Copulatory suckers are absent, but a pair of prominent copulatory cones are present lying lateral to the anus. Genital aperture is transverse between coxae IV. Body size, 504–595 μ in length including capitulum, and 336–430 μ in width. The body is 1.4 times as long as broad and the average dimension is 547 μ by 378 μ.

Rostrum (Fig. 9) is bilobed and bears a single pair of minute setae. Through it the pharyngeal support is clearly visible. Chelicera (Fig. 2) is two-segmented, markedly chelate, strongly sclerotized, and usually projecting well beyond the capitulum, thus being readily discernible from both dorsal and ventral aspects. *Palpi* (Fig. 9) very inconspicuous, superimposed on the ventral surface of the chelicerae and appressed to the rostrum. Each palpus is composed of two small free segments, moderately sclerotized, bearing three short setae.

Legs (Figs. 1, 8) attached to the venter; anterior pair of coxae separated from the posterior pair. Legs I and II are directed forward and outward, whereas legs III and IV extend out horizontally, leg III facing anteriorly, leg IV posteriorly. All legs are of similar length, —two thirds that of the body. Legs I and II are of equal width, but are stouter than legs III and IV, which are also of equal width. Epimera I are united in a V-form. Coxae I and II as well as III and IV of each side are united. Only coxa III is complete. Legs are composed of six segments; coxa, femur and tibia being the longest, while the tarsus is exceptionally small. The broadest segments comprise the coxae, trochanters and femurs I and II. A single prominent seta is present on the trochanters, femurs I and II, and two on each of the tibia. Small inconspicuous setae are located on patellae I to III,—three on I and II and a single one on patella III. Legs are practically devoid of sclerotized supports, except to a small degree in trochanters I to III.

Tarsi (Figs. 3, 4) consist of a small lobe bearing a pair of claws, a stalked ambulacrum and from three to nine setae. The claws of tarsi III and IV are equal in size and arise ventral to the ambulacrum. However, the more medial of the member of the pair of claws on tarsi I and II is markedly larger and stronger than the other and placed dorsal to the ambulacrum. The ambulacrum is flask-shaped, and borne on a non-segmented peduncle that is approximately 80 μ in length and arises slightly before the tip of the tarsus.

Body setae (Figs. 1, 8) are hair-like and of varying length. *Dorsum,* six pairs; consisting of two thoracic (an inner minute one and an outer one 40 μ in length); three small lateral setae grouped together; and one small postero-lateral one. *Venter* with 11 pairs of setae,—two epimeral (one on coxae I and II); three genital; five anal and a single postero-lateral seta. The genital setae are arranged as two anterior and one posterior genital seta. The anal setae comprise two that are antero-lateral and three that arise on the copulatory cones. The anterior genital, anterior anal and postero-lateral are from 12–20 μ in length; those of the coxae, posterior genital and the smallest seta on the copulatory cones from 28–36 μ; whereas the two long setae on the cones constituting the terminal setae measure as long as 274 μ.

Male (Figs. 10–12) differs from the female in body size, absence of conspicuous ambulacra and in the character of the genital and anal regions. The stalked ambulacra of the female are here represented by stumps, 20 μ long. The genital aperture is posterior to coxa IV and a prominent short curved penis is evident. The posterior end of the dorsum possesses a distinct sclerotized shield, and terminates as two lobes, equivalent to the copulatory cones of the female. Flanking the anus is a pair of strong circular copulatory suckers, 30 μ in diameter and separated from each other by a distance of 16.5–24 μ. The genital setae are small, not exceeding 12 μ in length. The small postero-lateral seta is located on the edge of the dorsum. Of the five anal setae, only one is antero-laterally placed with respect to the anus, the remaining four arise within the terminal lobe of the body. The two anal setae that are equivalent to the terminal setae of the female measure not longer than 75 μ in length compared with 274 μ of the female; the other three anal setae are approximately 12 μ long. The male is somewhat smaller, measuring 410–540 μ in length and 300–380 μ in width, with an average dimension of 467 μ by 339 μ.

Type material: Female holotype from the nasal cavity of *Larus delawarensis* Ord. at Galveston, Texas collected through March 14 to 25, 1947. Holotype and paratypes deposited in the United States National Museum.

Remarks.—The mite is described from 11 specimens, type female and 10 female paratypes. An egg is present within one of the females. It is oval measuring 291 μ by 197 μ with the more pointed end directed anteriorly. Its long axis is parallel to

that of the female and it extends from the level of the anterior edge of coxa III to the copulatory cones. Another female in the collection contains a well developed larva. The larva is 370 μ in length, extending from the level of the thoracic setae to the extreme posterior end of the parent's body, and is 260 μ in width. It is orientated so that its anterior end lies towards the posterior extremity of the parent. Its copulatory cones are inverted in position, and the legs face medianly,—legs I and II folded forward pointing backward and leg III backward then inward. There is no sign of the presence of an egg shell enclosing the larva. One of the three larvae in the collection had been evidently forcibly expelled by the bursting of the rear end of the parent in the preparation of the slide. It is 364 μ by 213 μ in dimension and the copulatory cones are still in the reverse position from what occurs in the free larval stage. Again there is no evidence of the presence of an egg shell—this may signify that the species is viviparous.

One of the seven nymphs was in the process of molting into a female when it was killed. The outline of the presumptive female is clearly defined within this nymph. Its legs and posterior end including the anus and copulatory cones are folded under its venter in such a way that the terminal setae of the cones are in contact with tarsi I and II. Legs I and II are curved forward and backward around the capitulum, while legs III and IV are bent backward then forward so that both sets of ambulacra interlock.

The genus *Turbinoptes* n.g. differs from all related genera in the form of its tarsus, which is very short, bearing two claws in addition to an ambulacrum, pedunculated in the female but vestigial in the male. A certain similarity in body structure exists between *Turbinoptes strandtmanni* n. sp. and members of the Psoroptidae, in particular the female of this species with the male *Psoroptes*. This is evident in their body shape, dorsal shield, long stalked ambulacra on all four tarsi, and copulatory cones with terminal setae. However, the family Psoroptidae is characteristically parasitic on mammals, and tarsi III of the female *Psoroptes* ends in whip-like setae instead of ambulacra. *Turbinoptes* n. g. has been placed in the family closely affiliated to the Psoroptidae, namely the Epidermoptidae (whose members typically parasitize birds). *Turbinoptes strandtmanni* n. sp. somewhat resembles *Microlichus uncus*, that belongs to the Epidermoptidae, and was collected from the skin and flight feathers of the English sparrow (Vitzthum, '34). In the female of both species ambulacra are present on tarsi I to IV and the epimeral and terminal setae are similarly arranged. However, the vulva of *Microlichus* is at the level of epimera II and there is only one large claw on tarsi I, one small one on tarsi II and claws are absent on tarsi III and IV. *Turbinoptes strandtmanni* n. sp. may represent a connecting link between the two families, Epidermoptidae and Psoroptidae.

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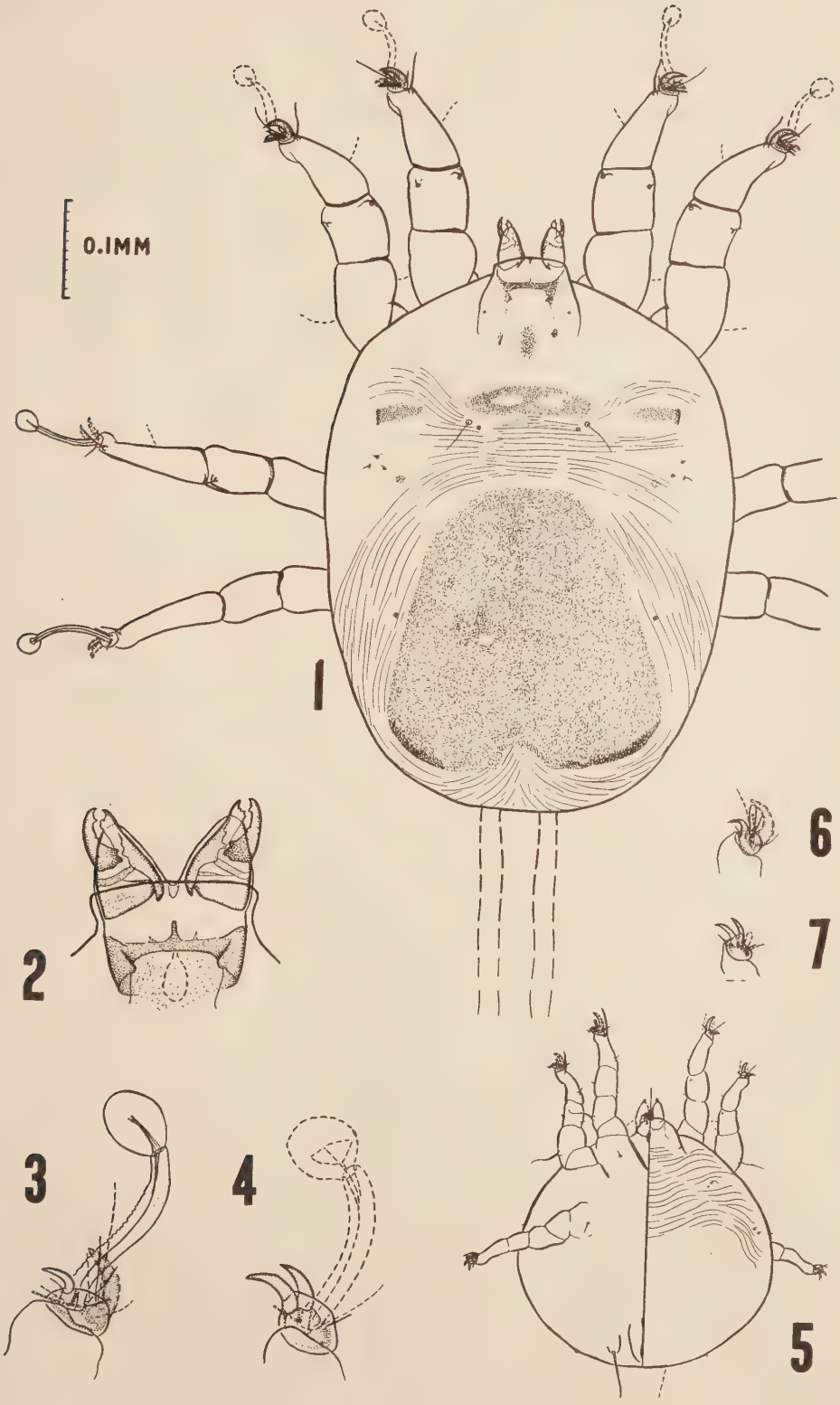
Explanation of Plates

Fig. 1. Dorsal view of mite; female.
Fig. 2. Chelicerae, sclerotized support of chelicerae and pharynx have been included.
Fig. 3. Venter of tarsus I; female.
Fig. 4. Venter of tarsus IV; female.
Fig. 5. Larva: entire, with right dorsal and ventral half of body illustrated.
Fig. 6. Venter of tarsus I; larva.
Fig. 7. Venter of tarsus III; larva.
Fig. 8. Ventral view of mite; female.
Fig. 9. Venter of rostrum to show palpi and sclerotized portions of palpi and pharynx.
Fig. 10. Genital region of male.
Fig. 11. Venter of tarsus II; male.
Fig. 12. Venter of tarsus III; male.
Fig. 13. Nymph: ventral view of posterior half of body.
Fig. 14. Venter of tarsus I; nymph.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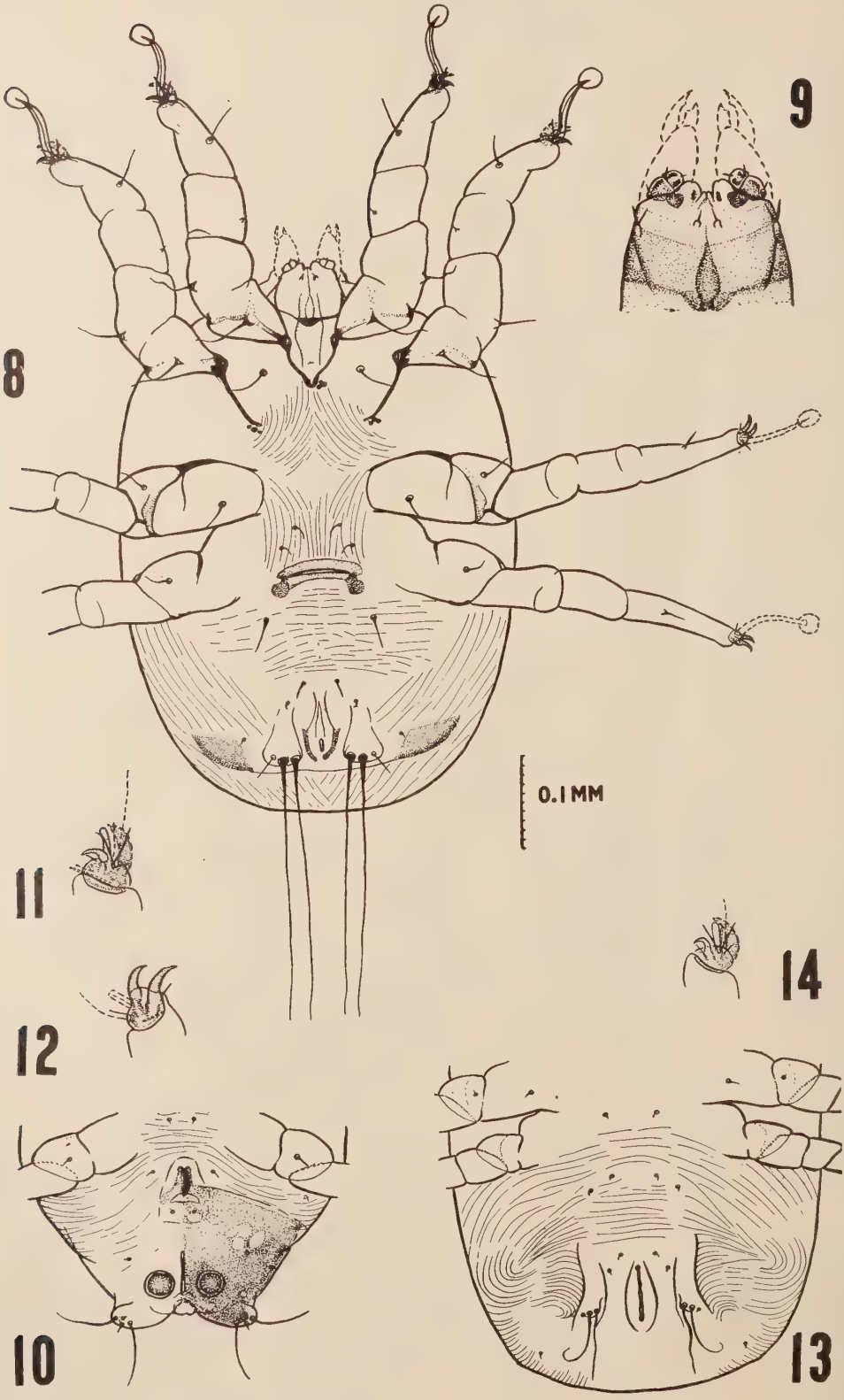
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ON A LARVAL NEMATODE FROM AN INSECT WITH A NOTE ON THE GENERA *THUBUNAEA* SEURAT, 1914 AND *PHYSALOPTEROIDES* WU AND LIU, 1940.[1]

M. A. Basir[2]

During the course of his investigations on parasitic nematodes of insects, the writer has found a few larval forms. As the insects serve as intermediate hosts for a large number of parasitic nematodes, it is thought desirable to publish an account of these larvae. In the present note only one of them is described; the others are in course of study and their description will be published later.

About fifty crickets were collected from cotton fields in Aligarh District (U.P.), North India and were examined for nematode infection. All were found to be free from infection with adult worms. However, the body cavity of one cricket was found to be almost filled by 130 free worms and some 35 in cysts of varying sizes. These larvae appear to be new and so far undescribed. On morphological examination they can be placed in the genus *Thubunaea* Seurat, 1914, but as much reliance cannot be placed on similarities between larvae and adults, it is thought proper not to group these larvae with any particular species of the genus already described or to give them the status of a new species. They may be referred to as the third stage or infective larvae of *Thubunaea* sp. from the body of an intermediate host.

Description: (Figures 1–5)

0.27	1.56	24.94	22.9	2.04	6.00 to 8.30 mm.
0.48	0.96	2.16	2.16	1.32	

The body length varies from 6.0 to 8.30 mm. In older larvae the cuticle is non-striated or the striae are so fine as to be hardly visible. In younger larvae fine striation is seen clearly throughout the length of the body. Lateral alae are present, extending from the head to the tip of the tail, and are about 5.3 to 7 μ broad.

The body tapers slightly towards the head which is truncate, its apparent raised structure being due to the lips. At the base of the lips the head is surrounded by a cuticular collar. A study of an *en face* view shows interesting characteristics (Fig. 2). The mouth opening is narrow and flattened, elongated dorso-ventrally. There are two asymmetrically developed pseudolabia or lips, the right lip is larger, is highly cuticularized and bears three well-developed teeth on its internal side and one tooth at its tip towards the outer side. All the internal teeth are almost similar in shape and size. The left lip is much smaller, and bears rudiments of three teeth which are neither well developed nor heavily cuticularized like those on the former. The lips appear to be capable of individual movement. There are four papillae on the outer margin of the head, more or less laterally situated; two of these are latero-dorsal and two latero-ventral in position. Amphids or lateral organs are present on the outer lateral sides of the lips.

The mouth cavity opens into a vestibule which is 22 μ long. The oesophagus occupies a length of about one-fourth of the body, being 1.85 to 2.07 mm. long. It is divided into two distinct parts, a short anterior one, 225 to 240 μ long by 25 to 30 μ broad, followed by a long posterior part which has a length of 1.62 to 1.83 mm. The latter gradually increases in diameter posteriorly. Anteriorly, it is about 60 to 70 μ broad while posteriorly it attains a breadth of between 75 and 100 μ. The whole of the oesophagus is traversed by a very narrow cuticularized oesophageal lumen. The granulations of the two portions are completely different.

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In the anterior portion the granules are finer while in the posterior portion they have an alveolar appearance. At its posterior end where it opens into the intestine, it bears a pair of valves, which appear to be cuticularized.

The intestine is fairly well developed and in older larvae a continuous lumen can be traced. It has an average width of about 70 μ. Posteriorly, it communicates with a short and narrow rectum, about 80 μ long, which is more or less hidden by the surrounding rectal glands. The latter are fairly well developed and measure between 100 to 105 μ in length by 30 μ in width. They apparently open into the rectum and are three in number. All the three glands are nearly of the same size. The anus is situated about 150 to 170 μ from the posterior end of the body. The tail is conoid in form. No papillae or spines are seen on the tail.

The nerve ring surrounds the middle of the anterior portion of the oesophagus and is about 130 μ from the anterior end of the body. Cervical papillae are spine-like and lie in the region of the nerve ring. The excretory pore is situated about 40 μ behind the nerve ring.

The sexes can be easily differentiated in these larvae. The females (Fig. 3) have already developed a complete outline of the reproductive apparatus. A vulva is formed as a circular opening with cuticularized margin in all the female larvae. It is situated about 30 μ anterior to the base of the oesophagus or about 23 per cent of the body length from the anterior end. The relative position of the vulva is almost constant in the worms studied. It leads into a vagina which bifurcates into the two uteri at about the level of the base of the oesophagus. The uteri have not yet developed a lumen. These extend posteriorly as ovaries which run parallel to each other and end blindly a little anterior to the middle of the body.

The male larvae (Fig. 5) can be easily distinguished from the female larvae by the form of the reproductive apparatus. In the former a testis is developed which extends anteriorly up to the base of the oesophagus where it ends blindly. It is not reflexed. Posteriorly it is connected with the rectum. The vas deferens and the ejaculatory duct have not yet formed a lumen.

Host: Gymnogryllus erythrocephalus.

Habitat: Body cavity.

Locality: Aligarh (U.P.), North India.

Remarks:

While most of the larvae of different sizes were found free in the body cavity of the insect, a considerable number of them was found coiled in cysts. The wall of the cyst (Fig. 1) is membraneous and its major portion is filled with the coils of the larva, the rest of its cavity being filled with globules and certain other dark substances. The cysts are nearly sperical in form and vary in size considerably, being 0.5 to 1.42 mm. in diameter. The larvae in smaller cysts form about two coils while in bigger cysts, larvae with three to three-and-a-half coils were seen. Some cysts were lying free in the body cavity of the host while others were attached to the intestine. Although nothing definite can be said at present about the origin and formation of the cysts, nevertheless it appears that the cyst wall has been elaborated by the intestine of the host. A few of the cysts were opened to examine the enclosed larvae. There appears to be no morphological difference between these larvae and those which were found free in the body cavity. Even the reproductive apparatus has developed to the same stage in both.

The laying down of the sex apparatus in these larvae is very significant. In cases where life-cycles of nematodes have been worked out, the infective larvae possess a genital rudiment consisting of a single cell or of several cells. In the larvae under consideration the development of the sex organs has progressed much further than has been observed heretofore in spiruroid larvae obtained from arthropod intermediate hosts. In spite of the advanced state of development of the sex organs, they have not yet reached the functional stage.

In studies of paedogenesis and neoteny it has been shown in animals other than nematodes that a delay or acceleration of development can be experimentally produced by alterations in environment and nutrition. In general, high temperatures would hasten sexual maturity and, overfeeding in some forms and hunger in others would result in a heterochronic growth. Termites specially fed have been shown to mature very early even while the wings are underdeveloped and the eyes have not yet appeared. A favorable parasitism is also looked upon as a factor accelerating maturity because of the abundant food supply. The present case may be considered as an example indicating at least partial paedogenesis or a forward step toward paedogenesis, the reasons for which are not clear. It might be due to a very favorable environment and to the supply of a highly suitable nutrition for the larvae growing in this particular insect. Whether the development of the sex organs to this stage in the third-stage larvae in the intermediate host of this species is a specific character or whether these have developed to this extent only in this isolated case, can only be decided after further study.

DISCUSSION

The pattern of the teeth on the lips and the form of the stoma easily place these larvae in the Family PHYSALOPTERIDAE. In the latter, asymmetrical lips have been described in only two genera. These are *Thubunaea* Seurat, 1914, and *Physalopteroides* Wu and Liu, 1940. The latter genus contains only a single species, *P. dryophisi,* which was collected from a snake in China. In its generic or specific diagnosis, the authors have not given anything about the presence or the absence of a vestibule in this worm. Their only reference lies in the statement: " . . . buccal cavity developed, distinctly seen in a lateral aspect of the worm." The description is accompanied by two diagrams of the anterior extremity of the worm. From one of these one can conclude that no vestibule is present while in the other the presence of a vestibule similar to that of *Thubunaea* is indicated. However, if a vestibule is present, the status of the genus *Physalopteroides* would become doubtful. Even if this point is set aside the larvae described here differ from *P. dryophisi* in the pattern of teeth on the lips. In the latter, the right lip bears a row of 5 teeth and the left lip has a smooth edge without any teeth.

The genus *Thubunaea,* which is characterized by a vestibule, at present contains 11 species, of which 6 possess symmetrical lips and the remaining 5 are with asymmetrical lips. The larvae under consideration can be compared with species having this cephalic asymmetry. *T. asymmetrica* Baylis 1930 was the first species with asymmetrical lips to be described and placed in this genus. After that, 4 more species with asymmetrical lips have been added to the genus. These are *T. agamae* Sandground, 1933; *T. grayiacola* Sandground, 1933; *T. dactyluris* Karve, 1938; and *T. impar* Malan, 1939. The first four of these species differ from these larvae considerably in the pattern of teeth on their lips. *T. asymmetrica* has three teeth on the left smaller lip, and the right larger lip bears no teeth at all. Both *T. agamae* and *T. grayiacola* have a pair of large and a pair of small teeth on the inside of each lip. The teeth are less developed on the left lip than on the right. In *T. dactyluris,* only the left smaller lip bears three teeth on its inner surface; the centre tooth is stouter and larger than the remaining two. In *T. impar* the left smaller lip bears on its inner surface three small forwardly directed teeth of which the central is somewhat larger than the laterals. The corresponding teeth on the right lip are larger. The latter also bears in addition, a strong tooth on the outside near the tip. In a dorso-ventral view the small teeth of the left lip are hardly seen, the teeth on the right lip being very conspicuous. A "cuticular groove" (collar) encircles the body at the base of the lips.

Thus the cephalic structure of *T. impar* is almost similar to that in the larvae under study. The position of the cervical papillae and that of the vulva is also the same in both. But it does not seem proper to depend merely on morphological similarities between the larvae and adults except when they are found in the same definitive hosts. Accordingly, it is thought advisable not to make these larvae cospecific with *T. impar.* Furthermore, the characters of the caudal end of the male and the size of the eggs are not available.

However, the finding of these larvae gives a definite clue in the right direction and will prove helpful in investigating the life-history which is not known for any member of this genus.

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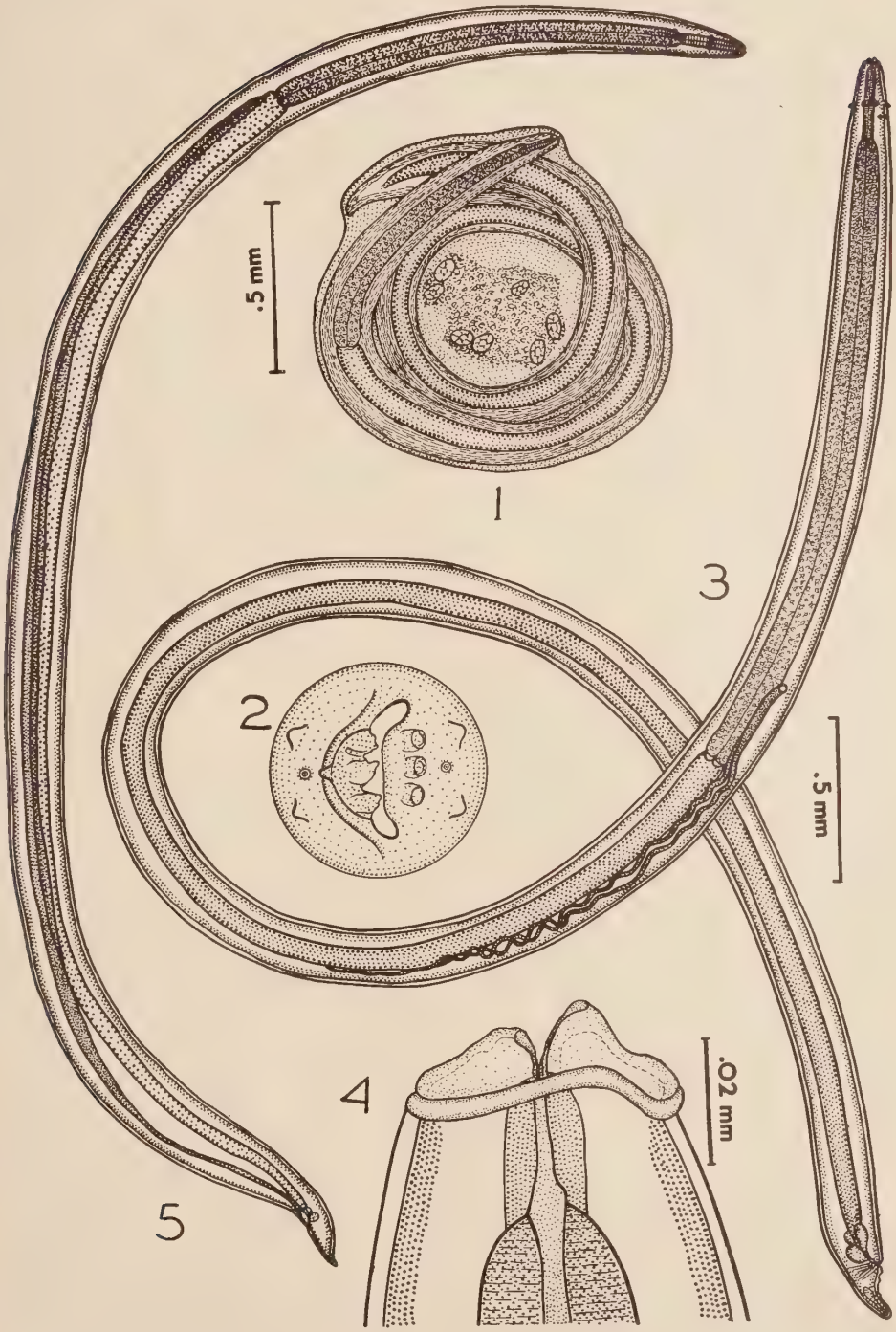

Fig. 1. Encysted larva, entire.
Fig. 2. *En face* view.
Fig. 3. Free larva (female), entire, lateral view.
Fig. 4. Anterior end, magnified.
Fig. 5. Free larva (male), entire, lateral view.

A CRITICAL STUDY OF NORTH AMERICAN CESTODES OF THE GENUS *ANDRYA* WITH SPECIAL REFERENCE TO *A. MACROCEPHALA* DOUTHITT, 1915. (CESTODA: ANOPLOCEPHALIDAE)

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Eight species of cestodes belonging to the genus *Andrya* Railliet, 1883, have been described from North American rodents. To the present time, 6 of these have been considered valid (*A. primordialis* Douthitt, 1915; *A. macrocephala* Douthitt, 1915; *A. neotomae* Voge, 1946; *A. microti* Hansen, 1947; *A. sciuri* Rausch, 1947; *A. ondatrae* Rausch, 1948). The genus *Andrya* was separated by Kirschenblatt (1938) into two subgenera, depending upon the presence or absence of a prostate gland. Of the North American species, only one (*A. primordialis*) possesses a prostate gland. Since we are concerned essentially with the other 5 closely-related species, *A. primordialis* is only briefly considered. The purpose of this paper is to present the data derived from a detailed study of morphological variation in certain of these cestodes, together with the resulting nomenclatural changes.

Testes number, testes distribution, and ventral excretory canal diameter have been hitherto considered of primary importance in the differentiation of new species. Testes distribution has been perhaps the most important single character used. In the past it has been considered possible to use these characters to separate the existing 5 species. This may be shown from the specific diagnoses, taken either from Baer's (1927) monograph, or from the original descriptions.

1. *Andrya macrocephala* Douthitt, 1915. Genital pores irregularly alternate. Ventral longitudinal excretory canals may attain a diameter of 320 μ. Testes 43 to 57 in number, situated anterior to the aporal half of the ovary, and extending beyond the aporal longitudinal excretory canals. Testes about 50 μ in diameter. Cirrus sac 160 μ long by 80 μ wide. Eggs measure from 30 to 32 μ in diameter.

2. *Andrya neotomae* Voge, 1946. Genital pores irregularly alternate. Testes 60 to 74 in number, confined to the space between the longitudinal excretory canals. Cirrus sac 320 to 444 μ long. Eggs measure 53 μ in diameter.

3. *Andrya microti* Hansen, 1947. Genital pores irregularly alternate. Ventral longitudinal excretory canals 25 to 41 μ in diameter. Testes from 28 to 35 in number, in aporal half of segment; occasionally a few may extend over on the poral half of the segment, but they never reach the midline of the ovary. Testes from 41 to 69 μ in diameter. Cirrus sac 178 to 233 μ long, by 55 to 69 μ wide. Eggs measure from 32 to 33 μ in diameter.

4. *Andrya sciuri* Rausch, 1947. Genital pores irregularly alternate. Ventral longitudinal excretory canals 30 μ in diameter. Testes from 100 to 110 in number, extending on both sides beyond the longitudinal excretory canals. Testes measure

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from 40 to 50 μ in diameter. Cirrus sac 200 μ long by 85 μ wide. Eggs measure from 52 to 56 μ in diameter.

5. *Andrya ondatrae* Rausch, 1948. Genital pores irregularly alternate. Ventral longitudinal excretory canals 85 μ in diameter. Testes from 75 to 95 in number, extending over both ventral longitudinal excretory canals, and sometimes beyond the aporal canal. Testes 66 to 86 μ in diameter. Cirrus sac from 213 to 224 μ long by 113 to 122 μ wide. Eggs measure from 33 to 39 μ in diameter.

The sixth North American species, *Andrya primordialis* Douthitt, 1915, is readily separated from the others by the presence of a prostate gland, and by the unilateral genital pores. One of us (R. R.) recently collected specimens referable to this species from *Phenacomys sp.* in Wyoming; it is noteworthy that in the case of these cestodes there was some irregularity in the position of the genital pores. At present we consider this only a variation.

It is obvious from the descriptions that *Andrya macrocephala, A. microti,* and *A. ondatrae* are morphologically very similar. The recent discovery of an unusual amount of morphological variation in the cestodes of the genus *Andrya* parasitic in voles from a southern Wisconsin marsh has indicated that some of these species should be restudied. With this in mind, we have attempted to secure all possible *Andrya* material.

As far as it was possible, our specimens were fixed under comparable conditions; they were first allowed to relax in water, and then were rapidly fixed by flooding with hot formalin-alcohol-acetic-acid solution. They were routinely stained in Semichon's acetic carmine,and sections were stained in haematoxylin-eosin.

One of us (R. R.) already had a considerable quantity of *Andrya* material; this had been collected over the past few years from the Ohio River north to central Manitoba, and from Pennsylvania west to Wyoming. Dr. E. W. Price kindly sent us all the *Andrya* slides in the Helminthological Collection of the U. S. National Museum. Dr. M. F. Hansen placed at our disposal several vials of unmounted specimens of *A. microti* from Nebraska. Miss Marietta Voge loaned slides of *A. neotomae.* Dr. Reinard Harkema sent us several slides of *Andrya* sp. collected from *Sigmodon* in North Carolina. Miss Darhl Foreman provided us with a specimen of *A. primordialis* from *Tamiasciurus* in Wyoming, one of the hosts from which it was first described. Messrs. N. C. Negus and J. S. Findley made available for examination the fresh carcasses of a large series of mammals collected in the Jackson Hole region of Wyoming. Mr. Merle Kuns placed at our disposal a considerable amount of *Andrya* material from Wyoming voles. We wish to take this opportunity to thank these persons for their cooperation, without which this study would not have been possible.

RESULTS AND DISCUSSION

Of the North American species of *Andrya, A. macrocephala* is the most common species east of the Rocky Mountains, and occurs in a wide variety of hosts. It is an extremely common parasite of voles (*Microtus spp.*) and was first recorded from *M. p. pennsylanicus* Ord by Rausch (1947). We have examined over 700 voles of this species from the North Central States region, and data on incidence and seasonal fluctuation resulting from these studies have been given in a previous paper (Rausch and Tiner, 1949). Although practically absent from voles (at least in the North

Central States) during the winter months, this cestode reaches a peak of abundance during late July and early August. An incidence of infection of 25 per cent was observed for 64 voles examined during the month of August, 1948, from an isolated southern-Wisconsin marsh. None of the 19 animals taken after August 17 was infected, although an infected animal was taken on September 4, and another on September 8. Some of the general parasite-host ecology of this area has been discussed in a previous paper (Rausch and Tiner, 1949).

The discovery of rather extreme variation in the cestodes infecting this ecologically-isolated colony of voles (plate I, figs. 1–4) forced us to look to other characters for the separation of species of the *macrocephala* group. Cestodes were collected which showed variation in testes number and distribution from what is seen in *Andrya microti* (plate I, figs. 7–8) to that seen in *A. ondatrae* (plate I, fig. 14). In fact, testes distribution was in some cases less extensive than that seen in *A. microti* (plate I, fig. 1). On the basis of this character alone, it would have been possible to describe one or more species. A cestode from this area, collected early in 1948, was previously reported (Rausch and Tiner, 1949) as *A. microti* Hansen.

Since *Andrya macrocephala* is the common species infecting voles in the North Central States region, it was assumed that variation in this species might be much greater than hitherto recorded. The present study has shown this to be the case—it is entirely unsatisfactory to attempt to differentiate *A. macrocephala* by testes number and distribution. Moreover, it has become evident that testes number and testes distribution, along with ventral excretory canal diameter, are of little value as specific characters unless they occur in combination with other characters which are relatively constant.

Table I presents data on the variation of certain characters in *Andrya macrocephala*. An effort has been made to include measurements from cestodes taken over a wide geographical area, and from a wide variety of hosts.

It seems pertinent to mention the lack of eggs in the uteri of the terminal segments of certain strobilae. Although the strobila may be of normal size, and of normal development otherwise as far as can be determined, there is often a partial or complete lack of eggs. In the case of complete absence of eggs, the segments may be much elongated. When no eggs at all are present, a given cestode often cannot be identified with certainty. This sterility is common in *Andrya macrocephala*, regardless of host species from which the specimens are taken. That only one of 6 cestodes examined from *Sigmodon* contained eggs in the uteri might be taken as evidence of an unnatural host-parasite relationship. However, this situation is rather commonly observed in voles, which we consider a natural host. Although the reason for this lack of egg development is unknown, in our opinion it is not a manifestation of parasite-host incompatibility. Lack of egg development has been noted by other writers (Douthitt, 1915; Hansen, 1947).

In view of the results obtained, it becomes necessary to invalidate two species—*Andrya microti* Hansen, and *A. ondatrae* Rausch. While these species could be considered quite distinct as long as the previous lack of knowledge concerning variation in this genus prevailed, they must now be considered identical with *A. macrocephala* Douthitt. It is unfortunate that new species must so often be described without adequate material to allow for some concept of the variation involved; how-

ever, the difficulties in this regard are usually such as to preclude a more desirable course of procedure.

It seems justifiable now to consider *Andrya caucasica* Kirschenblatt, 1938, identical with *A. macrocephala*. It is a parasite of voles, and the measurements of the differential characters fall well within the range determined for *A. macrocephala*. A final decision cannot be made until more information, and material for comparison, is available. *Andrya caucasica* has been recovered from the Transcaucasus region of Russia; with this possible exception none of the species considered in this paper has been recorded outside North America.

The problem of separating the remaining species of *Andrya* becomes more complex, as will the differentiation of any species to be described in the future. A thorough study of our material has not disclosed any single character which could

TABLE 1.—*Variation of certain characters in* Andrya macrocephala *Douthitt, 1915*

Host	Locality	Egg size		Testes no.		Testes distribution
		range	av.	range	av.	
Sigmodon h. hispidus	North Carolina	26–33	31 μ	66– 86	77	from aporal edge of ovary across aporal excretory canals.
Ondatra z. zibethica	Ohio	33–39	35 μ	75– 95	83	across entire field, over excretory canals on both sides; sometimes beyond aporal canal.
Thomomys talpoides tenellus	Wyoming	29–33	31 μ	24– 40	33	from poral edge of ovary across aporal excretory canals.
Microtus p. pennsylvanicus	Ohio	33–37	33 μ	39– 54	46	from poral edge of ovary across aporal excretory canals.
Microtus p. pennsylvanicus	Wisconsin	30–43	34 μ	48–106	64	from aporal margin of ovary to excretory canals; across entire field, between excretory canals, sometimes beyond aporal canal; or any distribution between these extremes.
Microtus pennsylvanicus drummondii	Manitoba	sterile		.....	..	from poral edge of ovary across aporal excretory canals.
Microtus ochrogaster	Illinois	30–36	33 μ	.....	..	from middle of ovary to aporal excretory canals.
Microtus ochrogaster	Nebraska	31–39	33 μ	37– 67	52	from middle of ovary across aporal excretory canals; from aporal margin of ovary across aporal excretory canals; also any distribution between these extremes.
Microtus townsendii	Washington	33–39	35 μ	46– 64	55	from middle of ovary across aporal excretory canals.
Overall averages and ranges		26–43	33 μ	24–106	59	

be relied upon, although it would seem that average egg size is of particular value. The 3 remaining species of the aprostate group may be characterized as follows:

Andrya macrocephala is recognized by an average egg diameter of 33 μ (range: 26 to 43 μ), and by the testes usually overlapping the longitudinal excretory canals on the aporal side only. The ventral longitudinal excretory canals may be much enlarged, and when this occurs it appears to be characteristic. However, this feature may often be lacking and is perhaps dependent upon the physiological state of the worm.

Andrya neotomae is recognized by a much larger cirrus sac (320 to 444 μ long), and by the fact that in combination with this the testes are confined to the area within the longitudinal excretory canals. Egg size serves to separate it from *A. macrocephala*, but not from the following species. The eggs range from 40 to 63 μ in diameter, with an average of 53 μ.

Andrya sciuri is recognized by the testes overlapping the longitudinal excretory canals on both sides, in combination with an average egg size of 52 μ (range: from 43 to 56 μ). It is readily separated from *A. macrocephala* by average egg size.

Other characters not mentioned in this paper, such as scolex size, sucker development, size of strobila, etc., are of little value in separating the species of *Andrya*. The scolices of the species considered are figured in plate II; figures 1–5 represent the variation seen in *A. macrocephala*. Mature segments of all the North American species are figured in plate I; figures 1–9, 12, and 14 represent *A. macrocephala*—an effort has been made to show details of worms from a variety of host species.

It should be pointed out that, while the original descriptions in all cases were consulted, our conclusions are based on data resulting from the examination of actual material. The types of all the North American species of *Andrya* were secured, and in all cases additional material was studied.

We conclude that no variation is brought about by the host species in which these cestodes occur. Size of host animal also does not seem important. While the specimen of *A. macrocephala* from the largest host (muskrat) was larger in size than is usually seen, specimens of equal size were taken from voles. There was no indication that multiple infections had any effect on the size of the worm—however, rarely more than 2 or 3 worms are taken from a single host, according to our observations. Although we have not had the opportunity to study *A. macrocephala* in *Geomys,* the host from which it was described, the infections observed by Douthitt (1915) in this host were light.

There appear to be local cestode "populations" which are fairly uniform. For example, the voles near Lansing, Michigan, and Columbus, Ohio, are parasitized by cestodes which are very similar to the typical *Andrya macrocephala* as described by Douthitt. The southern Wisconsin specimens are extremely variable. Those in voles near Lincoln, Nebraska, appear to be morphologically rather uniform, and were described by Hansen (1947) as a separate species. It might be mentioned that except for the single specimen from the Ohio muskrat, also described as a separate species (Rausch, 1948), cestodes with unusually great testes distribution were taken only from the Wisconsin area.

Included below is a host list for the 4 North American species of *Andrya*. A number of host- and distribution records not previously published have been included. New records are marked by an asterisk (*). Subspecific determinations of certain hosts are not included, since they were not given in the original papers.

Andrya primordialis Douthitt, 1915.
(Syn. *A. communis* Douthitt, 1915)

SCIURIDAE: *Tamiasciurus hudsonicus ssp.*—Minnesota; *T. hudsonicus ventorum* Allen*—Wyoming; CRICETIDAE: *Phenacomys i. intermedius* Merriam*—Wyoming; *Clethrionomys gapperi galei* (Merriam)—Colorado; *Microtus p. pennsylvanicus* Ord*—Ohio, Michigan; *M. pennsylvanicus modestus* (Baird)*—Wyoming; *M. montanus nanus* (Merriam)*—Wyoming; *M. montanus* ssp.—Washington; *M. longicaudus* ssp.—Washington; *M. richardsoni macropus* (Merriam)*—Wyoming.

Andrya macrocephala Douthitt, 1915.

(Syn. *A. translucida* Douthitt, 1915; (?) *A. caucasica* Kirschenblatt, 1938; *A. microti* Hansen, 1947; *A. ondatrae* Rausch, 1948).

GEOMYIDAE: *Geomys bursarius* Shaw—Minnesota; *Thomomys talpoides tenellus* Goldman*—Wyoming; CRICETIDAE: *Sigmodon h. hispidus* Say and Ord*—North Carolina; *Microtus p.*

pennsylvanicus Ord—Ohio, Michigan, Wisconsin; *M. pennsylvanicus drumondii* (Audubon and Bachman)—Manitoba; *M. pennsylvanicus modestus* (Baird)*—Wyoming; *M. ochrogaster* (Wagner)—Illinois, Nebraska; *M. townsendii* (Bachman)*—Washington; *M. townsendii pugeti* Dalquest*—Washington; *M. richardsoni macropus* (Merriam)*—Wyoming; *Ondatra z. zibethica* Linnaeus—Ohio; *Microtus socialis satunini* Ogn.—Tbilsi (Georgia) Russia; *Cricetulus migratorius* Pall.—Tbilsi (Georgia) Russia.

Andrya neotomae Voge, 1946.

CRICETIDAE: *Neotoma fuscipes* ssp.—California.

Andrya sciuri Rausch, 1947.

SCIURIDAE: *Glaucomys sabrinus macrotis* Mearns—Wisconsin.

SUMMARY

1. A critical study of morphological variation in *Andrya macrocephala* Douthitt, 1915, has been made.

2. Testes distribution, testes number, and ventral longitudinal excretory canal size are considered to be of no value *per se* in the differentiation of the North American species of *Andrya*.

3. Average egg diameter appears to be a reliable character, especially in combination with the above-mentioned characters.

4. *Andrya microti* Hansen, 1947, *A. ondatrae* Rausch, 1948, and probably *A. caucasica* Kirschenblatt, 1938, are considered to be synonyms of *A. macrocephala* Douthitt, 1915.

5. A host list, including new host- and distribution records, is included for the 4 remaining North American species of *Andrya*.

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Plate I

Mature segments of th North American species of *Andrya,* with special reference to the variation seen in *A. macrocephala.* All drawings original, made with aid of a projector. The scale has a value of 1 mm.

Explanation of Plate

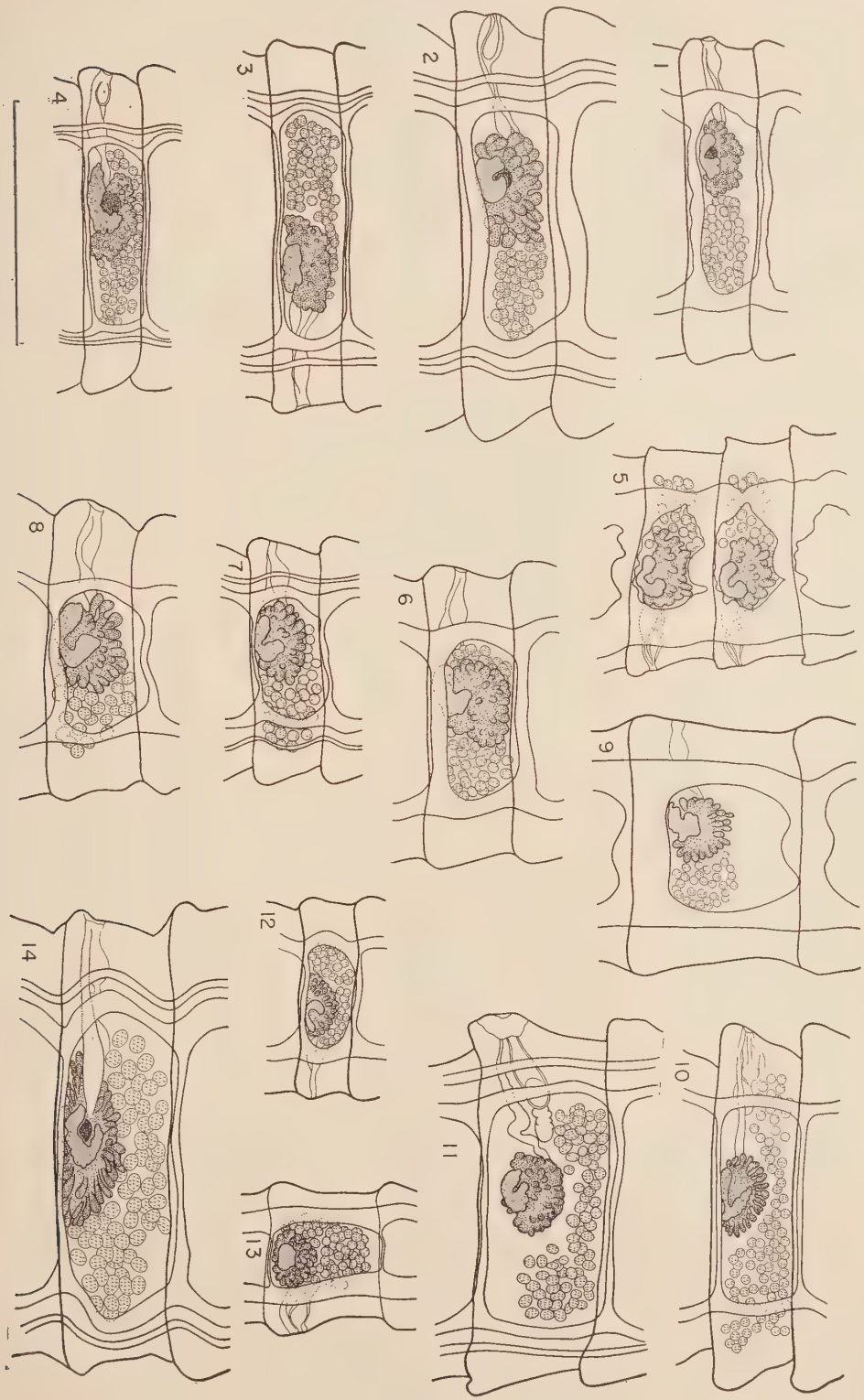
Fig. 1. *Andrya macrocephala* from *Microtus* (southern Wisconsin).
Fig. 2. *A. macrocephala* from *Microtus* (southern Wisconsin).
Fig. 3. *A. macrocephala* from *Microtus* (southern Wisconsin).
Fig. 4. *A. macrocephala* from *Microtus* (southern Wisconsin).
Fig. 5 *A. macrocephala* from *Microtus* (Illinois) ; similar to material upon which Douthitt's *A. translucida* was based.
Fig. 6. *A. macrocephala* from *Thomomys* (Wyoming).
Fig. 7. *A. macrocephala* from *Microtus* (Nebraska).
Fig. 8. *A. macrocephala* from *Microtus* (Nebraska).
Fig. 9. *A. macrocephala* from *Sigmodon* (North Carolina) ; from a strobila distorted by over-extension.
Fig. 10. *A. sciuri* from *Glaucomys* (Wisconsin).
Fig. 11. *A. neotomae* from *Neotoma* (California).
Fig. 12. *A. macrocephala* from *Microtus* (Manitoba).
Fig. 13. *A. primordialis* from *Microtus* (Wyoming).
Fig. 14. *A. macrocephala* from *Ondatra* (Ohio).

Plate II

Scolices of the North American species of *Andrya,* with special reference to the variation seen in *A. macrocephala.* All drawings original, made with the aid of a projector. The scale has a value of 1 mm.

Explanation of Plate

Fig. 1. *Andrya macrocephala* from *Microtus* (southern Wisconsin).
Fig. 2. *A. macrocephala* from *Microtus* (Ohio).
Fig. 3. *A. macrocephala* from *Microtus* (southern Wisconsin).
Fig. 4. *A. macrocephala* from *Microtus* (Nebraska).
Fig. 5. *A. macrocephala* from *Ondatra* (Ohio).
Fig. 6. *A. primordialis* from *Tamiasciurus* (Wyoming).
Fig. 7. *A. neotomae* from *Neotoma* (California).
Fig. 8. *A. sciuri* from *Glaucomys* (Wisconsin).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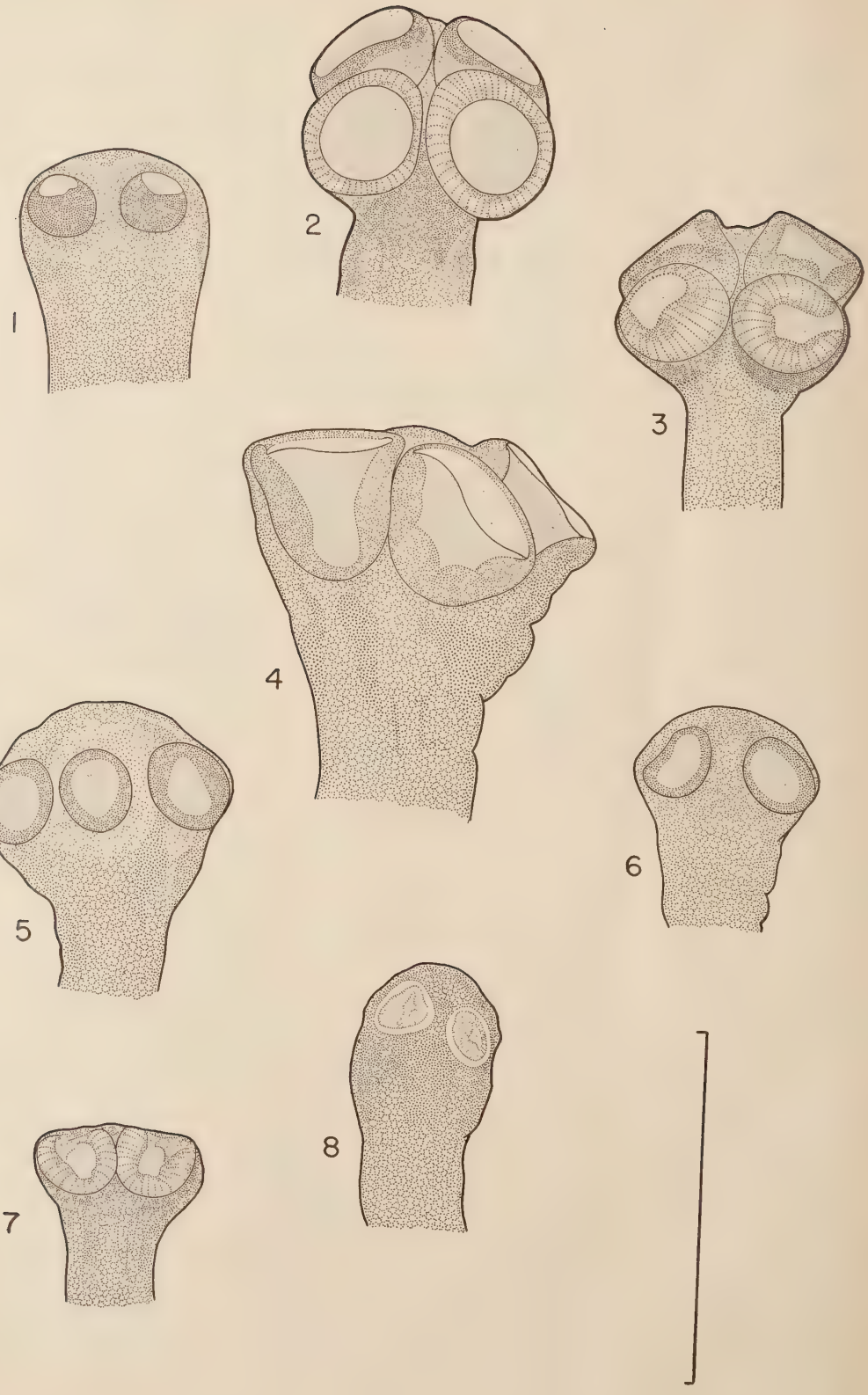
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STUDIES ON BOVINE GASTRO-INTESTINAL PARASITES XII. ADDITIONAL INFECTION EXPERIMENTS WITH THE HOOKWORM (*BUNOSTOMUM PHLEBOTOMUM*) IN THE CALF

Roy L. Mayhew
Louisiana State University

A preceding paper of this series (Mayhew 1948b) reports experiments in which 5 calves were inoculated by placing the infective larvae on the skin, 3 inoculated by mouth, and observations are recorded on 3 naturally infected animals. Since severe symptoms and one death resulted from the cutaneous inoculations, it seemed advisable to carry out additional experiments in order to study further the importance of and the variations in the results of this manner of infection. In the following pages are recorded the results of 9 additional experiments in which the larvae were placed on the skin. Since a discussion of the literature dealing with this subject is given in the former paper of this series (Mayhew 1948b), references are omitted in the following pages.

METHODS OF PROCEDURE

The methods of procedure employed in the following experiments were essentially the same as those used in the former inoculations (Mayhew 1948b). The source of the experimental animals, methods of feeding and management were the same, and the calves were fastened securely in stanchions so that they could not lick themselves until eggs appeared in the manure, or until 2 months after, except as stated. The larvae were applied over a wider area than in the preceding experiments in order to reduce the severity of the local irritation. Calves will usually show indications of irritation within 5 to 10 minutes after the application of the larvae by switching the tail, twitching the skin, kicking, and licking the affected area. Often there will be an exudate of lymph over portions of the area during the next day or two, followed by the formation of scales and scabs among the hair. In some instances patches of hair 1 to 3 inches in diameter have been shed. The calf usually shows more or less irritation for as long as two weeks by being itchy over the area of application and by switching his tail, kicking, and licking objects within reach. At no time when the skin irritations, diarrhea, or other disorders were observed in the inoculated animals were symptoms of any sort noted in the uninoculated animals fed and cared for in the same manner.

EXPERIMENTAL RESULTS

Calf No. 164. This animal was a pure bred Holstein male born November 15, 1946. On December 16, 1946 infective hookworm larvae were placed on the skin of the back and entire right side. The usual skin irritation developed during the next few days, followed by scale formation. A chalky-white diarrhea developed on January 8, 1947, but, in so far as was observed, his appetite remained good. On February 8, two to three hookworm eggs were found in each of the four fecal exami-

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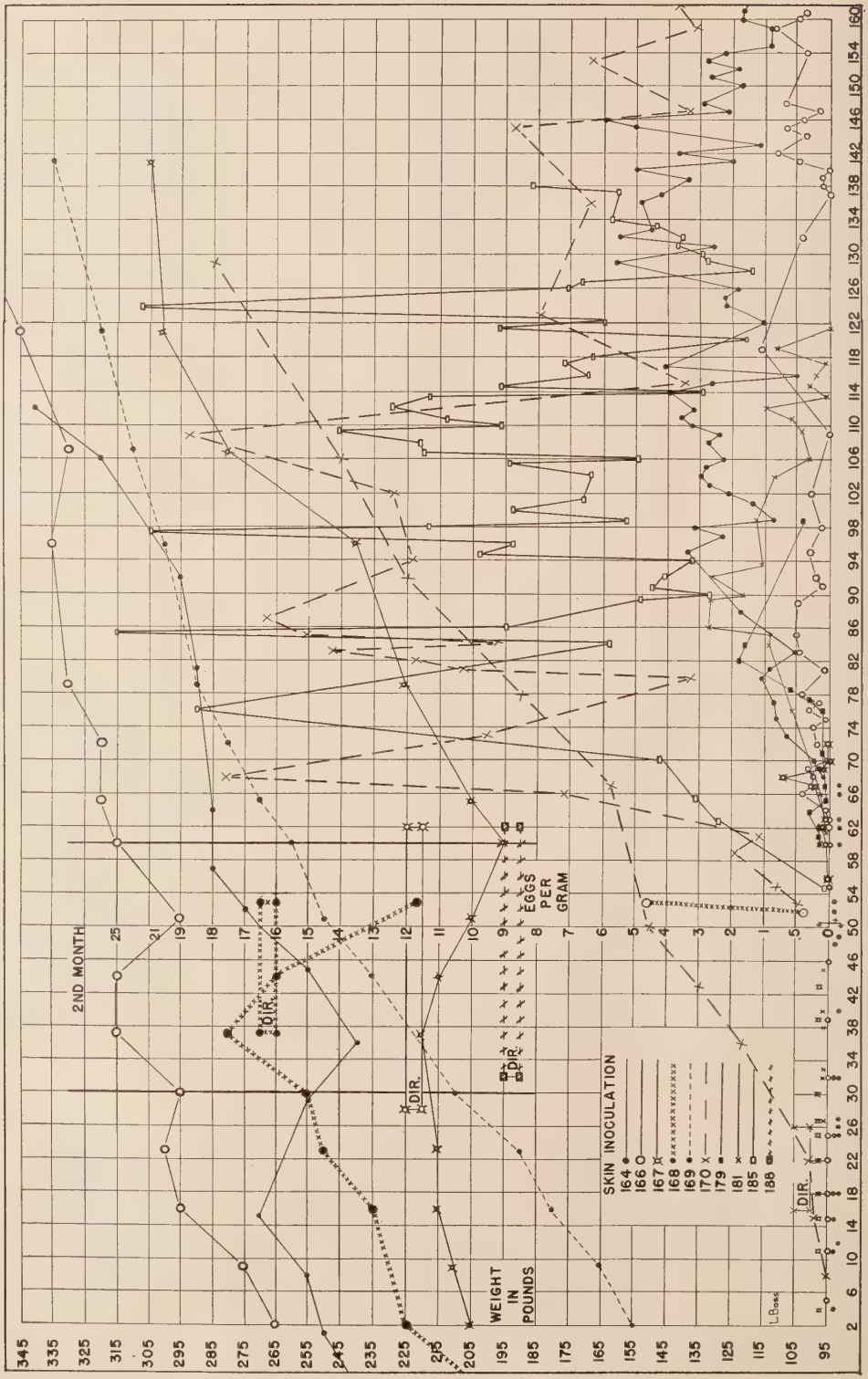
2ND MONTH
DIR.
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WEIGHT IN POUNDS
EGGS PER GRAM
SKIN INOCULATION
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181
185
188
L. Boss

nation slides and on February 14 one degenerated hookworm egg was observed. These were the only eggs observed in the 31 fecal examinations made between February 2 and March 15.

Additional inoculations were made by placing larvae on the skin on May 9, 15 and 19, 1947. Some skin irritation was observed but no acute symptoms of parasitosis, such as diarrhea, etc., were observed during the remainder of the experiment except that there was a loss of 30 pounds in weight between the 15 and 36th days after the first of these inoculations (fig. 1). There was a gain of 15 pounds during the next 9 days followed by increases as indicated to the end of the experiment. The first eggs appeared in the manure on the 68th day after the May 9th inoculation and the egg counts varied between 0.5 and 6 eggs per gram of sediment during the remainder of the experiment (fig. 1).

Calf No. 166. This animal was a pure bred Holstein male born December 18, 1946. Larvae were placed on his skin on April 24, 1947 and within 5 minutes the usual indications of irritation were manifested, followed by itchiness and scale formation during the next week. He was kept fastened in a stanchion so constructed that he could not lick himself from the time of inoculation until June 14, 51 days after inoculation. On June 25, 1947 the first eggs appeared in the manure 62 days after the application of the larvae. It will be noted (fig. 1) that the counts remained relatively low, all except 5 were below 0.5 eggs per gram of sediment. No symptoms of parasitosis, such as diarrhea, loss of appetite etc., were observed. It will be observed (fig. 1) that the weights showed regular gains during the first 23 days after inoculation. During the next week there was a loss of 5 pounds and during the following month, the second month after inoculation, the weights were irregular with a net gain of 15 pounds. During the following 60 days there was a gain of approximately 30 pounds and during the next 28 days a gain of 25 pounds.

Calf No. 167. This animal was a pure bred Jersey male born January 2, 1947. Infective hookworm larvae were placed on the skin on April 27, 1947. He was at once placed in a stanchion but escaped on May 21, 27 days after inoculation. A very severe diarrhea began on May 22, 28 days after inoculation, and continued until June 25, 62 days after inoculation, becoming less severe about June 11. On June 7 his appetite began to decrease and during the following two weeks he ate relatively little hay and at times refused the grain concentrate or ate only a portion and refused or drank only a portion of the regular feed of milk. There was a marked loss of strength and general condition. During the first 37 days after inoculation there was a gain of 15 pounds but during the next 23 days, the second month, there was a loss of 25 pounds in weight. This was followed by a steady gain in weight of 110 pounds during the next 70 days and there was a marked improvement in appetite, strength, and general condition during this period. The first eggs appeared in the manure on the 56th day after inoculation. It will be observed (fig. 1) that eggs were recovered from the manure of this animal only between the 56th and the 72nd days (June 19 to July 5) after inoculation, 8 positive fecal examinations, all relatively low, having been obtained. Following this, 19 negative fecal examinations were obtained between July 7 and September 22.

Calf No. 168. This animal was a pure bred Holstein male born January 25, 1947. On May 1, 1947 infective hookworm larvae were placed on the skin of both

flanks and the calf fastened in a stanchion until eggs appeared in the manure. Diarrhea was first observed on June 7 (fig. 1), 37 days after inoculation, and it continued until the time of his death on June 23, the 53rd day after inoculation. His weights also began to decrease with the onset of diarrhea. Beginning on June 13, the 43rd day after inoculation, his appetite became progressively poorer and after June 20 he refused to eat altogether. On the morning of June 23, 53 days after inoculation he was unable to get up or stand when helped to his feet and died between 12 noon and 1 P. M.

At the postmortem examination the 4th stomach appeared normal except possibly some of the folds showed slight oedema. The wall of the anterior 12 feet of the small intestine was normal in appearance. The wall of the next portion of the small intestine back to a point 20 feet anterior to the ileocaecal valve was very pale in color except for very numerous pinhead-size red spots, presumably the points of attachment of the worms. The posterior 20 feet was pale and lacked the red spots. The red color of the contents of the small intestine indicated the presence of much blood. The walls of the large intestine were normal in appearance except very pale in color. The contents of the caecum were very fluid and red in color while the contents of the remainder of the large intestine became progressively firm due to the absorption of water toward the rectum where it was nearly firm at the time of death. Blood was not observed in the discharge of this animal during the period of acute symptoms. A total of 4993 hookworms were recovered from the anterior 2/3 and 401 from the posterior 1/3 of the small intestine. Two positive fecal examinations only were obtained, these on the 52nd and 53rd days after inoculation as indicated in fig. 1.

Calf No. 170. This animal was a pure bred Holstein male born on May 21, 1947. On May 23rd larvae were placed on the right side from the flank to the neck and the usual skin irritations were observed during the next two weeks. Between June 8 and 17, beginning 15 days after inoculation there was a slight diarrhea and on June 6 he refused about ½ the usual feeding of milk. Since this period of diarrhea occurred much earlier than in any of the other inoculated animals it may have been due to bacterial infection, however, none of the other animals on the same diet showed any diarrhea. It will be noted that he made steady gains in weight throughout the experiment. The first eggs appeared in the manure on the 53rd day after inoculation and the resulting egg counts were among the highest in this series of experiments (fig. 1).

Calf No. 169. This animal was a pure bred Holstein male born February 21, 1947. He was never inoculated and served as a control for the other animals used in these experiments. It will be observed (fig. 1) that he made regular gains in weight and a total of 15 fecal examinations made between June 9 and October 15, 1947 were all consistently negative.

Calf No. 179. This animal was a pure bred Jersey male born October 10, 1947. Infective hookworm larvae were placed on the skin of both sides on February 18, 1948 and he was fastened in a stanchion until March 18. The skin reactions were so severe in this animal that the hair was lost on several areas 2 to 3 inches in diameter. Hookworm eggs first appeared in the manure on April 18, 60 days after the application of the larvae. The egg count remained relatively low, the maximum obtained was only 1.8 eggs per gram (fig. 1). The eggs disappeared altogether after May 27,

7 negative counts having been obtained between May 28 and June 11, 1948. No diarrhea or other symptoms of parasitosis were observed and, although weights were not taken, the calf seemed to make reasonably good gains.

Calf No. 181. This animal was a pure bred Jersey male born October 30, 1947. On February 4, 1948, hookworm larvae were placed on the skin of the back and both sides from the flank to the shoulder. He was fastened in a stanchion until the first eggs appeared in the manure on April 6, 1948, 62 days after inoculation. The number of eggs recovered by fecal examination was never high, the maximum being 2.81 eggs per gram of sediment (fig. 1). The egg count gradually decreased and after June 6, 1948 the following five daily examinations were entirely negative. No symptoms of parasitosis were observed, and, although no weights were taken, the animal seemed to make about normal gains.

Calf No. 185. This animal was a pure bred Jersey male born November 24, 1947 and was inoculated on February 4, 1948, the larvae being placed on the skin of the back and both sides from the shoulder to the flank. He was placed in a stanchion until the first eggs appeared in the manure on March 29, 1948, 54 days after inoculation. The number of eggs recovered by fecal examination from this animal was relatively high, a maximum of 25.32 having been obtained on April 29 (fig. 1) and, although the numbers gradually decreased somewhat, the high range was maintained until July. During July there was a rapid decrease and on the 28th the eggs disappeared altogether; the following 6 daily examinations were negative. No symptoms of parasitosis were observed, and, although weights were not taken, the calf seemed to make reasonably good gains throughout the entire period of the experiment.

Calf No. 188. This animal was a pure bred Holstein male born January 20, 1948. He was inoculated by placing the larvae on the skin of the left side from the shoulder to the flank on June 4, 1948. Evidences of skin irritation were observed in about 5 minutes followed by marked itching and scale formation during the next 10 days. He was fastened in a stanchion before placing the larvae on the skin and kept fastened so he could not lick himself until July 2. From July 4, the 31st day after inoculation, until August 8, the 62nd day after inoculation, the calf was observed to have a very severe diarrhea which varied somewhat in intensity from day to day. The discharge was grayish-white in color and very sticky but did not contain blood. From July 9 till August 7 the calf's appetite was very poor since he refused the grain concentrate and milk most of the time and ate very little hay. Following the end of the period of acute symptoms on August 8, he showed a rapid improvement in appetite and general condition until the end of the experiment on August 23, 1948. It was very apparent that the animal lost considerable weight during the period of acute symptoms. No diarrhea, loss in weight or in appetite were observed in any of the other animals kept under the same conditions of feeding and management at the same time. A total of 29 examinations for parasitic eggs, made between the time of inoculation and the 80th day after inoculation, were consistently negative for parasitic eggs except a few *Strongyloides sp.*

DISCUSSION

It is evident from the results described above that the degree of severity of the symptoms and the egg counts vary greatly when the different experiments are com-

pared. In general, the calves which exhibited the less severe or no symptoms developed the highest egg counts while those animals which showed the most severe symptoms had relatively low egg counts or remained negative. Examples of the former are Nos. 170 and 185 and of the latter Nos. 167 and 188. These same variations were observed in the animals reported upon in the former paper (Mayhew 1948b); No. 159 did not exhibit any symptoms and has a relatively high egg count while Nos. 162 and 163 showed severe symptoms and remained entirely negative. One possible explanation is that the conditions in the digestive tract become so abnormal that the larvae are unable to survive. It is also possible that an immunity is developed which causes the destruction of the larvae. It is also of interest to note that in the two animals that died, No. 168 (this paper) and No. 165 (Mayhew 1948b), relatively large numbers of hookworms were recovered. This indicates that some individuals are unable to combat the infection successfully and are overcome. These variations suggest that the calves in an infected herd that have shown the least symptoms are the greatest source of larvae which may result in new epidemics, and, therefore, should be treated to remove the adults. These extreme variations in the results of infection make diagnosis more difficult, since fecal examinations reveal low egg counts (rarely more than 20 eggs per gram of sediment while in stomach and nodular worm infections the egg counts are conmmonly very much higher). And also postmortem examinations result in the finding of relatively few adults due to the elimination of large numbers of larvae during the period of symptoms.

It is also evident from the above results that after the larvae reach the adult stage and begin producing eggs the animals begin to improve in general condition and increase steadily in weight. We have never observed any symptoms in our experimental animals during the adult life of the parasites. This observation also holds true for the animals in our experiments with the stomach worm and nodular worm, most of the results of which have been recorded in former papers of this series. Consequently, the general improvement in condition following the usual treatment for parasites is evidently due to the progressive recovery from the symptoms caused by the larvae and not to the removal of the adults. Treatment may be effectively used as a means of reducing the eggs and consequent accumulation of larvae about the barn, pasture, and lots which serve as the source of further infection.

The fact that cutaneous infection readily takes place, with the production of serious symptoms and death, adds the problem of barn and shade sanitation to that of the pasture in the management program. Cutaneous infection has been demonstrated in a total of 14 animals, 9 in the present paper and 5 in the former paper (Mayhew 1948b).

SUMMARY

The results of 9 experiments in which cutaneous infection with the cattle hookworm, *Bunostomum phlebotomum,* was demonstrated are recorded. Acute symptoms, loss in weight, and one death occurred between 30 and 60 days after inoculation. The first eggs appeared in the manure between the 52nd and the 68th day after inoculation. The degree of infection as measured by egg counts was not correlated with the severity of the symptoms, but the animals with the highest egg counts had

previously shown the slightest symptoms while several of the animals with low egg counts or were negative had shown severe symptoms.

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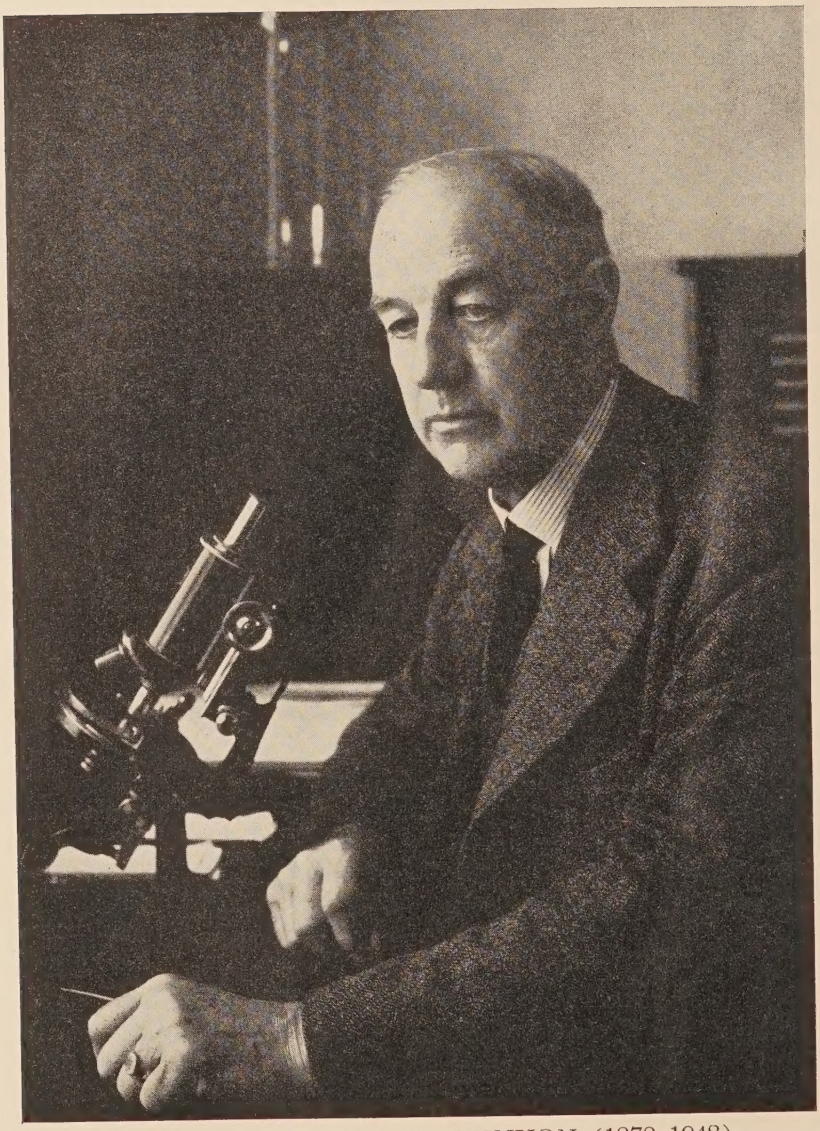
IN MEMORIAM



DR. CHARLES MORLEY WENYON (1878–1948)

By the death of C. M. Wenyon, October 24th, 1948, in London, the American Society of Parasitologists lost one of its most distinguished foreign members, many of its members lost a personal friend, and the science of protozoology lost one of its outstanding leaders.

Wenyon was born in Liverpool, March 24, 1878, the son of Dr. Charles Wenyon, a pioneer medical missionary. He spent his early childhood in China, but returned to England in 1892 for his education. His higher education began at Leeds Univer-

sity and continued at University College and Guy's Hospital, London (B.Sc., 1901 and M.B., B.S., 1904).

During World War I, he served as Lieutenant-Colonel, R.A.M.C. and, besides London School of Tropical Medicine (1904–1914), he started a career that included not only a unique contact with some of the great pioneers in tropical medicine and zoology but also exceptional opportunities to study protozoan infections. Thus, he was associated with Manson in London, Mesnil in Paris, and Richard Hertwig the Middle East and malaria in the Macedonian campaign.
East.

In 1914, he commenced his connection with the Wellcome Bureau of Scientific Research of which he became successively director of research in the tropics and director-in-chief in 1924. Later, he became director of research in the Wellcome Foundation. He retired in 1944, but remained a consultant in tropical medicine.

During World War I, he served as Lieutenant-Colonel, R.A.M.C. and besides other work, carried out his now famous studies of the human intestinal protozoa in the Middle East and malaria in the Macedonian campaign.

Wenyon was one of the small group of men who put medical protozoology on a firm basis during and following World War I. His work on the intestinal protozoa was of great importance in properly assessing the relative importance of bacillary and amebic dysentery, in understanding the epidemiology of amebiasis, and in analyzing the host-parasite relationships of the human intestinal protozoa. His contributions on trypanosomiasis and leishmaniasis were fundamental and on the latter group laid the basis for solving the problem of transmission of the parasite. His studies on malaria in Macedonia during World War I were crucial for understanding the military importance of the disease and contributed to our knowledge of its treatment and control. He was fundamentally interested in the relationship between man and his protozoan parasites, and combined with this a catholic interest in the zoology of the protozoa as a whole. His greatest service to science was his uniform ability to make sound basic conributions on a wide variety of subjects and to synthesize and critically evaluate the work of himself and others. This ability is well-illustrated by his "Protozoology." Published in 1926, this book immediately became the standard reference and arbiter of questions relating to the parasitic protozoa, especially as regards morphology, life cycles and classification and has become a classic in the field. Next to his "Protozoology" in influence are his reviews in the Tropical Diseases Bulletin. These reviews, in character with his other writings, reflect his sound judgment and tact. Wenyon was never unwilling to disagree or to criticize work which did not seem well grounded, but always managed to do it courteously. His kindness and charm were a constant source of pleasure to visitors from all over the world.

Wenyon was elected Fellow of the Royal Society in 1927. In addition, he received many honors from his own and other countries. In the United States, he was a foreign member of the American Society of Parasitologists, an honorary life member of the New York Academy of Sciences, and recipient of the Theobald Smith medal of the American Academy of Tropical Medicine in 1946.

William H. Taliaferro

CORRECTION

The mailing dates of the issues of Volume 33 (1947) were printed in the February number of Volume 34 (1948). The mailing dates of the December (1947) issue, Volume 33, No. 6, as published, are reversed. Section 2 of that issue appeared on December 12, 1947 and Section 1 on February 23, 1948.